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ERNST THEODOR von BRÜCKE
1880–1941

THE EDITORS of the *Journal* deeply regret to announce the sudden death of Ernst Theodor von Brücke on the night of June 11–12th. Dr. von Brücke had been a member of the Advisory Board of the *Journal*, representing neurophysiology in Austria, since the *Journal's* inception in January 1938.

Ernst T. von Brücke, born October 8, 1880 in Vienna, was for many years Professor of Physiology at Innsbruck, where he enjoyed the affectionate regard and high esteem of pupils and colleagues alike. As a teacher his eminence was attested by the crowded attendance at his lectures even when attendance was optional. In research he was a scholarly and impartial judge of evidence, a skilled technician and an eager and enthusiastic worker. Many important and fundamental papers on the nervous system came from his laboratory.

The honesty and sincerity of his quest for truth was shown by his readiness and even eagerness to accept criticism, to concede errors in interpretation and to revise his views as soon as new evidence indicated the need of revision. He wanted the truth regardless of whether or not it was at variance with his previous opinions.

A true scientist, he loved his work and his laboratory, and when on the Nazi conquest of Austria in 1938 he was dismissed from his professorship and ordered not to re-enter his laboratory, his depression was profound and pathetic. Enforced idleness and separation from the work he loved were almost more than he could bear. Even Nazi students, meeting him in the street, told him of their regret that they could no longer enjoy his teaching.

An appointment as Research Associate in Physiology in the Harvard Medical School was arranged for him, and he came to America in August 1939. Entering enthusiastically on a new program of research, he at once began to achieve noteworthy results in the study of the refractory phase of nerve and the effect thereon of prolonged stimulation—a problem which his earlier experiments with Field had rendered controversial and in need of clarifying. These researches have already resulted in the publication of one paper, while a second is in press in this *Journal*.

In recent weeks he had begun a new series of experiments on reflex inhibition, requiring a rare combination of skilled techniques. Significant results had already begun to appear. It was my privilege on June 11 to confer with him on these observations and to watch him performing one of the most brilliant experiments I have ever seen. He went home, apparently in the best of health and spirits, and happy in the progress of his new research. Early the next morning he died peacefully in his sleep.

High as was his standing as a scholar and investigator, he will be remembered chiefly by those who knew him for his extraordinarily kind and sympathetic nature and his nobility of spirit.

ALEXANDER FORBES

Harvard Medical School
Boston, Mass.
June 16, 1941

CEREBRAL BLOOD FLOW AND pH IN EXCESSIVE CORTICAL DISCHARGE INDUCED BY METRAZOL AND ELECTRICAL STIMULATION

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(Received for publication June 24, 1940)

INTRODUCTION

THE FUNDAMENTAL MECHANISM involved in the initiation of paroxysmal excessive discharges of the cerebral cortex has been investigated recently from several avenues of approach. Epileptiform discharges of the cerebral cortex, either experimentally induced by electrical stimulation or by convulsant drugs in animals, or as associated with epileptic seizures in man are accompanied by an increase in circulation through the discharging area, as shown by Gibbs (1933), Gibbs, Lennox, and Gibbs (1934), and Penfield, von Sántha and Cipriani (1939). The change in circulation appears, however, to be secondary to the increase in neuronal discharge. Since a change in cerebral circulation has never been observed to precede the onset of excessive neuronal discharge, but always to follow evidence of a convulsion, Penfield, von Sántha and Cipriani conclude that circulatory changes probably do not play a part in the immediate initiation of epileptiform activity. No direct evidence has been given pertaining to the mechanism of this increase in blood flow but the release of CO_2 or acid metabolites of activity has been suggested as well as a possible local accumulation of acetylcholine. Simultaneous records of local blood flow and the electrical activity of the cortex have not been reported.

Simultaneous measurements of electrical activity, pH and DC voltage changes of the cortex have led Dusser de Barenne and colleagues (1937, 1938 and 1939) to conclude that increased pH as well as a change toward negativity of cortical DC level are associated with facilitation of cortical response. Facilitation was manifest by increase excitability to electrical stimulation with increased after-discharge as well as increased spontaneous electrical activity.

Bonnet and Bremer (1937), Moruzzi and Bremer, (1938), Moruzzi (1938, 1939) and Miller, Stavraky and Woonton (1940), point out that acetylcholine may reproduce a condition of facilitation of the cortex similar to that caused by intense electrical stimulation. They suggest the possibility of acetylcholine playing a part in the normal facilitation process, or in the words of Bremer, (1938) to raise the "tonus corticale." Alterations in cerebral circulation have not been considered with these other factors for facilitation. The present study is concerned with the relationship between focal alterations in blood flow and pH with excessive cortical discharge in response to electrical stimulation and convulsant drugs.

METHOD

Experiments were carried out on 23 cats under light ether anesthesia with curare and artificial respiration or, in some experiments, with dial anesthesia. A bilateral craniotomy was performed exposing the right and left sigmoid gyri. Most records were taken from the posterior sigmoid gyrus of one side with electrical stimulation of the homologous area of the other hemisphere. Recording leads were applied immediately following the opening of the dura and special care was taken to maintain optimal conditions of temperature and humidity throughout the experiment.

FIG. 1. Schematic diagram of arrangement of electrodes on the cortex. The silver silver-chloride wick electrodes (W) are placed on either side of the glass electrode (G) with the thermocouple (T) placed so that its tip lies just beneath the glass electrode. The wick and glass electrodes are supported in a spring holder to minimize changes in pressure from movements of the brain.

The electrical technique for the simultaneous recording of focal cerebral blood flow, pH and electrograms together with femoral blood pressure has been described in a previous report (Jasper and Cipriani, 1940).* In addition to these measures a similar technique was employed in the present study for the recording of DC voltage changes at the site of the pH glass electrode. The disposition of the various recording devices on the cortex is shown in Fig. 1. Silver silver-chloride wick electrodes were used as non-polarizable leads for DC voltage measurements, one of the wick electrodes being used in common with the reference lead for pH measurement. This made it possible to record the DC voltage across an area of the cortex as nearly as possible the same as that used for the pH measurement. The wick electrodes were in contact with the glass electrode on either side, as shown in Fig. 1.

In preparing the cortex the dura was removed and the pia perforated or partially removed just beneath the point in the post sigmoid gyrus where the glass electrode was to be applied. This was found advisable for the pia caused some delay in the recording of cortical pH changes as determined by records taken before and after its incision. The thermocouple for blood flow was placed about 1 mm. beneath the surface of the pia with the point exactly beneath the glass electrode.

In order to estimate the amount of temperature correction for the pH measurement the thermocouple was used to measure cortical temperature in some of the experiments. Since, however, adequate temperature readings of the cortex immediately at the point of application of the pH electrode were not taken throughout all of the experiments, the absolute values of our pH measurements are subject to an experimental error estimated as of the order of ± 0.03 units. The temperature coefficient of our glass electrodes was 0.007 pH per degree C. and showed no significant variation from one electrode to another.

Control studies turning on and off the heater element of the thermocouple have shown that the heat of the thermocouple itself does not affect appreciably the pH measurements. This would also indicate that temperature changes beneath the pH electrode due to alterations in blood flow are probably insignificant in affecting pH measurements.

The latency of the glass electrode was determined by placing it in a phosphate buffer of pH 8.0 and injecting a drop of the same type of buffer pH 7.5 just beneath the glass electrode with a syringe. A second record was taken with an additional electrode in the solution to provide a signal for the instant at which the drops came in contact with the

* We are greatly indebted to Dr. Leslie Nims for teaching us the glass electrode technique as applied to the exposed cortex.

surface of the liquid. With injections immediately beneath the glass electrode, the latency of the recorded change in pH was about 0.08 seconds. Latencies as long as 0.2 and 0.3 sec. were obtained when a drop of the buffer solution was placed beside the glass electrode in the solution. The diffusion time to the glass electrode may add to this delay in actual experiments as demonstrated by these calibrations. No satisfactory measure of this factor was made. Its importance is hard to estimate. Electrograms were taken from as nearly as possible the same cortical area as for the pH measurements. Electrical activity may be recorded from deeper layers, however, causing a delay in pH of that area as recorded from the surface. The latency of the thermocouple for changes in temperature was determined by exposing a light to the tip of the thermocouple beside which was placed a photoelectric cell to signal onset of light. The latency to initial perceptible change was 0.17 sec. The amplifiers used for recording possessed sufficient in-phase degenerative feed-back so that completely independent records were obtained from each amplifier, as proven by repeated control tests.

Electrical stimulation was carried out by means of a thyratron stimulator at a frequency of 60 cycles per sec. through bipolar silver electrodes with a separation of 3 to 5 mm. Stimulation was always carried out on the contralateral cortex to avoid the effect of passing current through the recording electrodes. Both bipolar and monopolar leads were used for the electrograms either with separate silver electrodes or by using the same wick electrodes as for the DC measurements. The essential feature of the technique employed for all these experiments is that simultaneous records of focal changes in pH, blood flow, DC voltage and electrical activity were recorded in ink on a single strip of paper. Direct measurements of the temporal sequence of events was thereby made possible. In the experiment where DC voltage was substituted for blood pressure, blood pressure was read from the mercury manometer and inserted on the record by hand.

Experimental Results

Adrenalin

Effect on blood flow. Injection of adrenalin intravenously (0.2 to 0.4 cc., 1/10,000 solution) was used to test the reliability of the thermocouple for changes in blood flow. The invariable rise of blood pressure so produced was accompanied by a constant increase of blood flow through the cortex, thereby furnishing a good test of the sensitivity of the thermocouple as well as the direction of change.

Effect on electrogram. Under the conditions of our experiment there was either no perceptible change or a slight increase in the electrical activity of the cortex following the administration of adrenalin, if the animal had not previously received an injection of metrazol, and if the pH level of the cortex was relatively normal at the time of the injection (Fig. 2). Following an injection of metrazol, however, after the metrazol discharge had ceased, an injection of adrenalin would cause its reappearance. Also, if a dose of metrazol had been too small to produce large discharges of the cortex, the subsequent injection of adrenalin would precipitate the typical metrazol activity. This potentiation of metrazol discharge with adrenalin has been observed by previous investigators (Hall *et al.*, 1938).*

* Previous injection of adrenalin did not affect the convulsive threshold to metrazol. For this reason as well as because of the fact that adrenalin itself produces no such change in brain waves, it may be assumed that adrenalin mobilizes metrazol from some other organ which has taken it up. It has been shown that the liver is capable of retaining for some time large amounts of metrazol (Esser and Kühn, 1933). It is quite probable that metrazol taken up by the liver is mobilized into the blood stream by adrenalin analogous to the mobilization of glucose from glycogen stores.

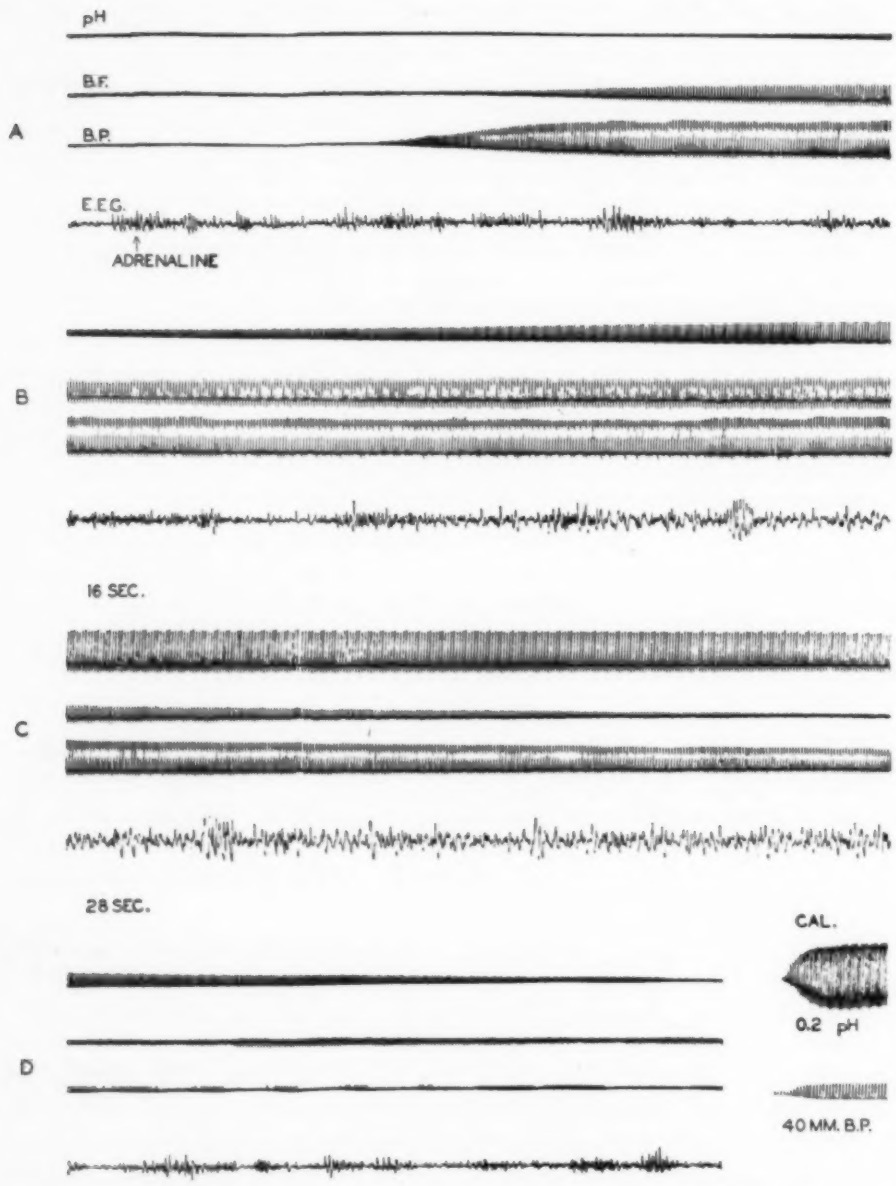

FIG. 2. The effect of adrenalin on focal cerebral pH, blood flow (B.F.), systemic blood pressure (B.P.), and cortical electrogram (E.E.G.). Initial pH balanced at about 7.2. Adrenalin injected into saphenous vein was followed in 9 sec. by a rise in B.P. and in 14 sec. by a rise in local cerebral blood flow (A). The pH did not begin to rise until about 25 sec. following the injection (B). Maximum pH rise was about 1.4 which was maintained after B.F. and B.P. had returned to normal (C and D). All records returned to preinjection level about 150 sec. following the injection (end of D). There was a moderate increase in the electrical activity of the cortex during the rise in pH. Each full line of record equals 30 sec.

Effect on pH of cortex. Under the conditions of our experiment the most common effect of adrenalin was to increase the pH of the cortex. This increase in pH usually followed the increase in blood flow, and often persisted longer than the increase in blood flow (see Fig. 2). The change in pH would seem to be a direct result of the removal of acid metabolites by the improved circulation.

Dusser de Barenne, Marshall and Nims (1938) demonstrated that the pH of the interstitial fluids of the cortex does not always follow exactly the pH of arterial blood. No records were taken of the pH of arterial blood but it seems logical to assume that, under most conditions, the cortical pH was somewhat below that of the blood so that an improvement in circulation tended to its increase. There were a few exceptions to this which probably signify that under certain conditions the balance between the pH of cortex and blood is so close than an increase in circulation causes little change in cortical pH, but the usual effect was an increase.

Metrazol

Intravenous injection of metrazol (pentamethylenetetrazol) was used as a convenient and dependable method of inducing abnormal and excessive discharge of the cortex. Even though the type of large amplitude electrical discharges obtained from the cortex, and the type of convulsions produced simulate those observed in certain forms of epilepsy, it cannot be assumed with certainty that the mechanism of the metrazol seizure is the same as that of epileptiform discharges produced by other methods.

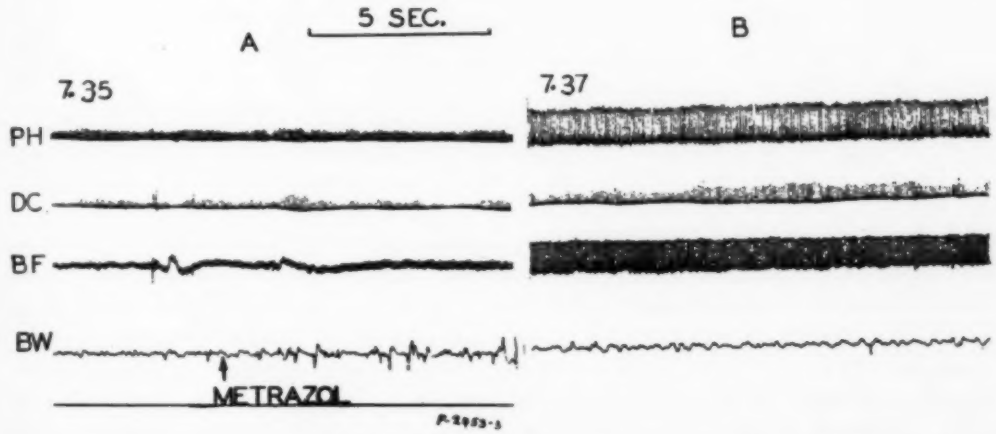

FIG. 3. Metrazol subliminal for cortical discharge but causing increase in blood flow and pH. In A, pH is shown (upper line) approximately balanced at 7.35 when metrazol (0.5 cc. of 10 per cent sol) was injected. DC voltage between the two wick electrodes was approximately balanced as shown in second line and blood flow (B.F.) on the third line. Electrical activity between the two wick electrodes (A.C. recording without "chopper") is shown in fourth line. Five minutes later (B) the pH increased to 7.37 with a small rise in blood flow (third line) and little change in D.C. (second line) or in electrical activity (lower line).

Metrazol was given by rapid intravenous injections in doses of 0.020 to 0.050 g. per kg. body weight.

Subliminal doses. Doses too small to affect cortical activity produced marked changes in blood pressure, blood flow, and pH. The increase in blood pressure was followed by an increase in blood flow and this, by an increase in pH (see Fig. 3). Further demonstration is here provided of the effect of increased blood flow on cortical pH, which must be kept in mind in evaluating changes due to supraliminal metrazol injections.

Supraliminal doses. With supraliminal injections of metrazol a sharp and large increase in electrical activity was in every instance the first ob-

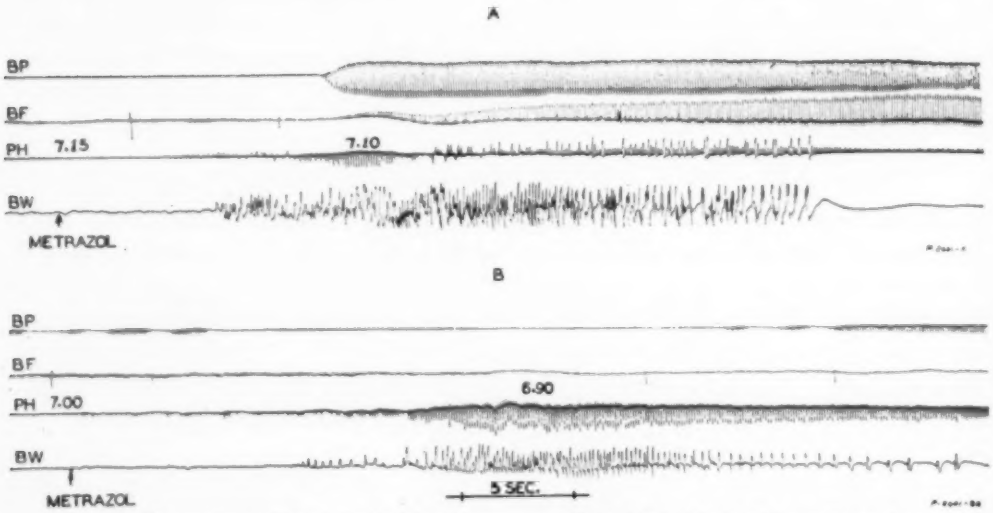

FIG. 4. Sequence of changes in blood pressure (BP), blood flow (BF), pH, and cortical electrogram (BW) following the injection of metrazol (0.6 cc. 10 per cent sol.). A. First injection, showing initial pH decrease (7.15 to 7.10) and compensation with pH increase as blood flow rises (blood pressure reaction not typical). B. Third injection about one hour later, showing only pH decrease (7.0 to 6.90) with no blood pressure or blood flow change.

servable change to occur. Following closely upon this initial burst of large amplitude waves there were changes in pH, blood flow and blood pressure. Changes in pH usually preceded slightly the change in blood flow in the majority of the most reliable experiments, but a simultaneous change in pH and blood flow as well as a slight precedence of blood flow change was observed in some instances. In a typical experiment the metrazol discharge appears in the electrogram 7–10 sec. following injection in the saphenous vein. About 2–3 sec. later the pH is decreased and blood flow begins to increase. Then the pH may return to normal or go relatively alkaline if there has been a sharp rise in blood flow.

The first pH change following the metrazol discharge was usually in the acid direction. If there was a prompt increase in blood flow the pH would revert to the alkaline side and then either return to the preinjection level, or go again "acid," depending upon the amount of compensatory blood flow

increase. With no marked change in blood flow, only an acid change in pH would be observed for the duration of the excessive neuronal activity. These changes are illustrated in Fig. 4A and B.

The initiation of the metrazol discharge appeared therefore to be due to an exciting or facilitating action of metrazol upon nerve cells apparently not related to initial changes in pH or blood flow. The latter changes appear as a result of excessive activity induced by metrazol. This is assuming a negligible diffusion lag in the pH system. The fact that the initial pH change is usually in an acid direction is a strong argument for its being due to the initial increase in neuronal activity. Some of the initial changes in blood flow may of course be due to direct effect of metrazol on the cardiovascular system. This would complicate the observed relation between pH and blood flow as related to metabolites from excessive neuronal activity.

There was an *increase* in cortical blood flow parallel to an increase in blood pressure following metrazol injection in the majority of experiments. Little change in blood flow or a decrease was observed only when the animal was in poor condition as when repeated doses of metrazol had been given, or the initial pH was low (7.0 or less) a condition often associated with low blood pressure. This has been confirmed by direct observations of cerebral vessels in experiments of Erickson and Dancey (unpublished).

There seemed to be a delicate interaction between blood flow and pH, apparently for the maintenance of constant pH of interstitial fluid in the presence of abnormal neuronal discharge. With prolonged and excessive cortical discharge this homeostatic mechanism would often fail to keep the pH at a normal level, so that a continued increase in acidity would result. After several metrazol injections the pH was not uncommonly found to go below 7.0. Values above 7.1 were maintained when the animal was in good condition.*

Spontaneous cyclic discharge. In many experiments, following the initial metrazol discharge lasting 10 to 20 sec.; cortical activity would suddenly disappear as though turned off with a switch. Then after a quiet period of 15–25 sec. it would suddenly reappear spontaneously, in all its former vigor. The duration of the active and quiet periods varied considerably but this periodic alternation in violent activity and quiescence might continue for over an hour without additional metrazol if the cortex was in good condition. This phenomenon afforded a good opportunity to study the relation between cortical pH and cortical activation.

Blood flow showed no periodic change associated with the cycles of electrical activity. There was usually a steady *increase* in blood flow at first until a maximum was reached.

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* The pH of the cortex was found to be a reliable index of the general condition of the animals. In animals which had suffered loss of blood or operative shock a low initial pH was always obtained. The death of the animals was often preceded by a continued decrease of pH well below 7.0 even though the electrical activity of the cortex was still present, though depressed.

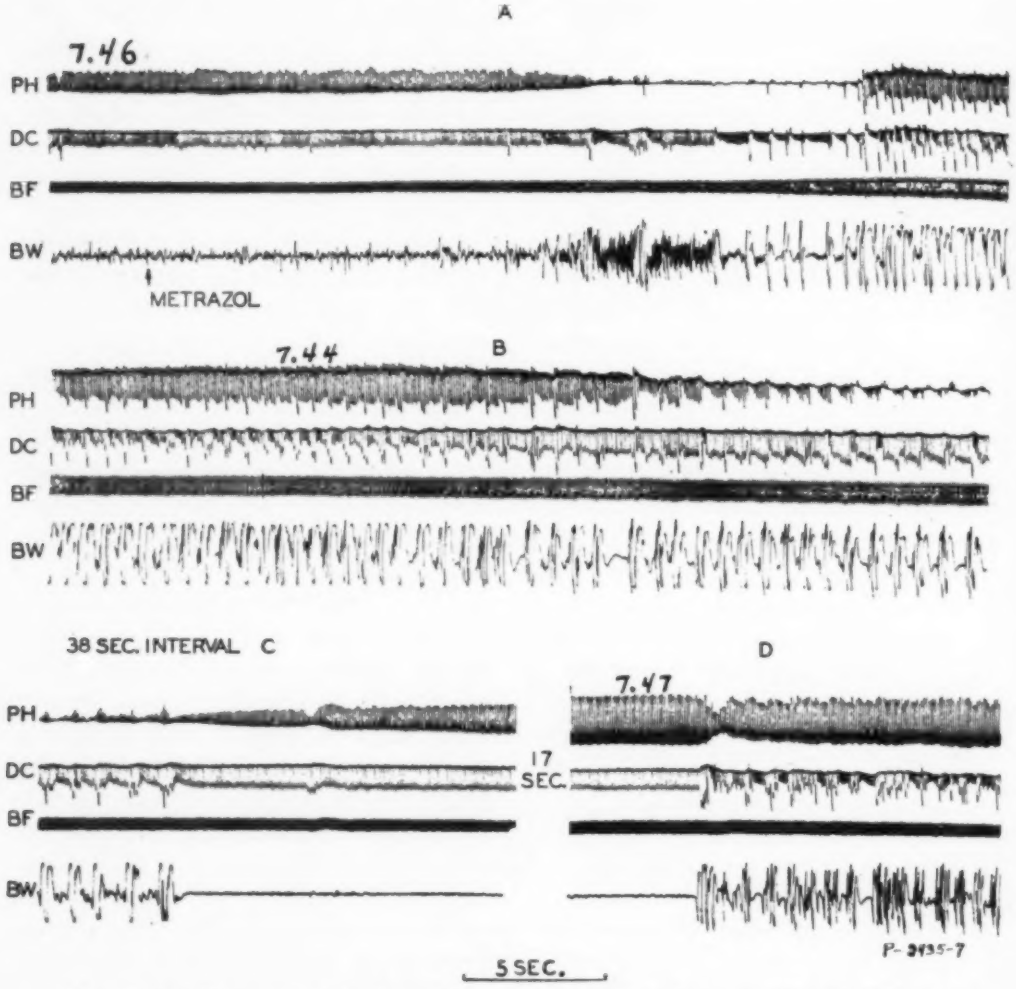

FIG. 5. Cyclic metrazol discharge (BW) with associated changes in pH. DC voltage, and local blood flow (B.F.). ("Chopper" records were not balanced completely at beginning.) Initial pH calculated to be about 7.46 before metrazol. Concurrent with the onset of metrazol discharge in lower line of A the pH dropped to reach 7.44 in B. With the increase in blood flow shown in third line (B) the "acid" drift of pH was arrested and partially compensated during continued metrazol discharge (end of B and for 38 sec. following). Then the metrazol discharge suddenly stopped spontaneously (C) after which the pH slowly rose to 7.47 when another prolonged metrazol discharge began to repeat the cycle. These pH changes seemed independent of DC voltage changes.

There was an acid drift in pH during the active phase of the cycle and an alkaline drift during the quiet period. If the blood flow was increased sufficiently during the active phase the pH would show an alkaline drift following the acid drift *during the cortical discharge*. The cortical discharge would suddenly stop, in this case, when the cortical pH was more alkaline than during the height of activity (see Fig. 5). Relative acidity could not be called upon to explain this sudden cessation of cortical activity. Change in pH seemed to

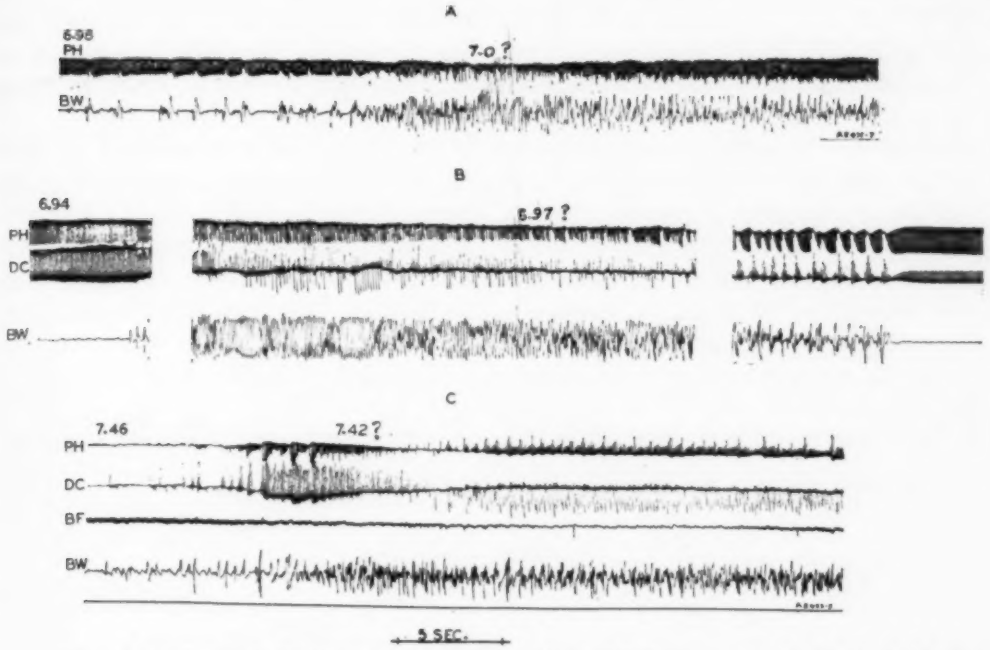

FIG. 6. Apparent "alkaline wave" due to artifact of DC component in cortical electrogram. A—Slight interrupted "alkalinity" with most rapid phase of electrogram. B—Change in DC simultaneous with apparent alkalinity of pH during rapid phase of electrogram and slow waves of "pH" at end due to slow DC changes in electrogram. C—Parallelism of DC and "pH" records during metrazol discharge. In this case the initial apparent "pH" change was "acid."

follow changes in blood flow but did not show any clear effect on the metrazol discharge. The cortical pH at any given moment was a function of the amount of neuronal activity which caused a decrease and the compensatory increase in blood flow which tended to counteract the developing "acidity."

After the cortical blood flow had reached its maximum the pH would then begin to show a steady acid drift throughout the interrupted trains of metrazol waves. If the cortical activity was continued long enough the pH might reach values below 7.0 before an appreciable diminution in the violence or duration of periodic discharges was detected.

Initial "alkaline waves." An apparent initial "alkaline wave" with the onset of metrazol discharge was observed in a few experiments, confirming the observation of Dusser de Barenne and McCulloch (1939). This initial "alkaline wave," when it did occur, seemed to be related to DC voltage components of the large cortical discharge. In some instances it may have been associated with DC polarization changes. An example of such an alkaline wave is shown in Fig. 6A and B. It will be noted that the alkalinity is associated with the most rapid phase of cortical discharge.* With the more rapid

* Dusser de Barenne and McCulloch (1939) also associate the "alkaline wave" with the rapid phase in electrocorticograms but interpret this as meaning greater facilitation. It appears to us that this might also produce a greater voltage artifact.

recording system used, the pH record shows an irregular interrupted appearance. With a slow moving galvanometer recording the pH changes, this would appear as a smooth alkaline wave. It seems, therefore, to be an artifact due to electrical disturbances of cortical origin.

In order to control this factor, simultaneous records were taken of the DC voltage changes from two wick electrodes placed on either side of the glass pH electrode, using one wick for the common reference electrode of the pH system as well. As shown in record B of the above figure, there was occasionally a definite relationship between DC voltage and changes in pH. This was not always in an alkaline direction however, as illustrated in Fig. 6C. It depended upon whether the DC voltage change occurred nearer the reference electrode or nearer the glass electrode, there being an equipotential point between them.

Further control on the DC voltage artifact was made by disconnecting all the leads except those measuring pH to rule out the possibility of interference between the recording systems. The voltage artifacts appeared even though only pH records were being taken, which showed that they originated at the cortex and were not in the recording apparatus. Also, when a 1–3 per sec. alternating current signal was placed on the two wick electrodes instead of the brain potentials, variations in the pH record could be induced artificially by voltage changes comparable in amplitude to those produced by the brain itself during a metrazol discharge. The DC artifacts were of the order of 1 to 2 mV. in magnitude or an equivalent pH of 0.02 to 0.04 units. We believe that these few observations of a small initial alkalinity with the onset of metrazol discharge can be explained on the basis of DC artifacts.*

The late alkaline waves, mentioned above, following an increase in blood flow are of larger amplitude (up to 0.36 pH units with large increase in blood flow) than could be produced by DC artifacts and are apparently related to the increased circulation. Changes of this order of magnitude were observed by the action of adrenalin alone.

Electrical stimulation

It was not possible to determine with accuracy the sequence of pH and blood flow changes following electrical stimulation, due to the large artifacts introduced into the records by the stimulating current. Records of blood pressure and blood flow were devoid of this artifact, but the pH and brain potential records were affected because of their high input resistance. Our observations will be confined mostly to changes associated with after-discharge phenomena following intense stimulation.

We were able to confirm the evidence of von Sántha and Cipriani (1937)

* It must be kept in mind that the alkaline waves observed by the Yale investigators were in response to monobromide of camphor rather than metrazol, and were present in animals under dial anesthesia. It appears clear however from our experiments that initial alkalinity played no part in the onset of the metrazol discharge. Also the occasional "alkaline waves" observed by us were small when not associated with increased blood flow, a factor not controlled in the Yale experiments.

that there is a focal increase in cerebral blood flow following local activation of the cortex from nerve impulses conducted over neuronal pathways from a distant area subject to electrical stimulation. In some instances this focal change in blood flow due to indirect stimulation was followed by a change in blood pressure (see Fig. 7). When there was marked increase of blood flow the pH either showed very little change or a slight alkaline drift. Following several intense stimulations, either with or without prolonged electrical after-discharge, the change in blood flow would be slight and the pH would show an acid drift increasing with successive stimuli. The acid drift was more marked when a long after-discharge was present.

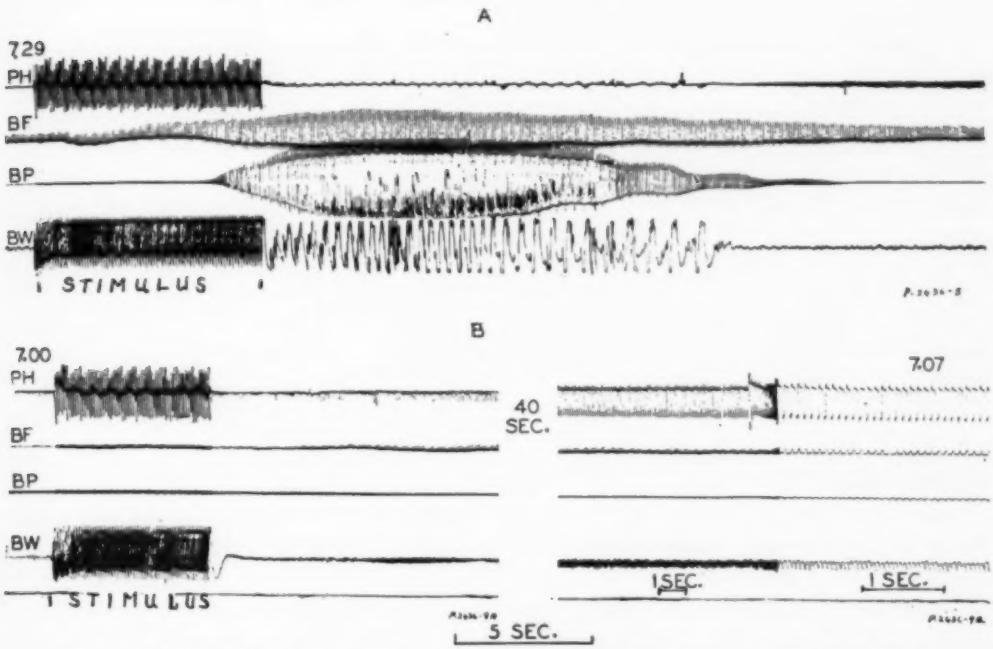

FIG. 7. Electrical stimulation of left post sigmoid gyrus with records of pH, blood flow, and cortical electrogram from the right post sigmoid gyrus. Blood pressure (BP) is from the femoral artery. Stimulus artifact appears in pH and cortical electrograms only. A—Stimulus at normal pH with rise in blood flow and blood pressure followed by after-discharge. The increased blood flow is adequate to maintain a constant pH in this example. B—Stimulus at low pH causing low voltage high frequency after-discharge, increase in blood flow and pH.

A consistent alkaline drift in pH was noted in a few experiments in which the after-discharge was of an unusual form, namely low amplitude, decelerating, rapid waves, as shown in Fig. 7B. In this example the initial pH of the cortex was low so that even a slight increase in blood flow was apparently sufficient to produce an alkaline drift of the pH. This low amplitude discharge may be associated with the production of less acid metabolites than the more violent discharge. Here again pH and blood flow changes appear to be results of local activation through nerve impulses from the

contralateral hemisphere. Changes in pH and blood flow do not seem to play an essential role in the *initiation* of abnormal neuronal discharge.

Local application of strychnine and metrazol

Local application of 3 per cent strychnine solution warmed to approximately the temperature of the cortex produced the usual strychnine spikes from the area to which it was applied. These spikes first appear as isolated random discharges separated by an interval of several seconds. During these isolated random spikes no significant change in either blood flow or pH was observed. Similar results were obtained in one experiment with the local application of metrazol solution at the same concentration as used for intravenous injection.

Records of blood flow were not reliable in these experiments due to the possible change in temperature following local application. It is of interest however, that strychnine spikes can be obtained with no significant change in pH, suggesting that here also the excitatory effect of strychnine is independent of pH changes. This is in confirmation of the work of Dusser de Barenne and associates.

Induced changes in pH

The pH of the cortex was altered by hyperventilation, apnea, intravenous injection of 5 per cent solution of sodium bicarbonate in normal saline, intravenous injection of 1/10 normal HCl, and by inhalation of a 15 per cent CO_2 in oxygen mixture.

The extreme changes in pH were found to alter the spontaneous activity of the cortex in the direction indicated by Dusser de Barenne and his colleagues (1939).

Large amplitude brain waves have been observed to follow the intravenous injection of sodium bicarbonate associated with a rise in the pH to slightly over 7.5.

Low amplitude brain waves were regularly observed without metrazol when the pH dropped to the vicinity of 7.1 or below. There were only small changes in spontaneous electrical activity of the cortex with variations of pH between 7.1 and 7.5. Large amplitude metrazol activity was observed with variations in pH between below 7.0 to slightly above 7.5. Injections of HCl in the midst of metrazol discharge, sufficient to alter the cortical pH as much as 0.2 to 0.3 units did not alter significantly the continued discharge, if the pH did not fall much below 7.0. With pH values below 7.0, however, there was a tendency in some of the experiments for the metrazol activity to be of lower amplitude and of short duration. Since other factors varied with this low pH, such as poor cerebral circulation, a depression of the metrazol discharge with pH below 7.0 cannot be certainly attributed to a change in pH alone.

In marked contrast to the lack of effect of pH on the metrazol discharges, apnea was followed within 10–30 sec. by a complete arrest of electrical activity of the cortex even though induced in the midst of violent metrazol

discharge. Apnea was always followed by an acid drift of the cortex, but the abolition of neuronal discharge occurred 20–40 sec. before the change in pH. This delay in pH change does not seem entirely due to diffusion lag for it is longer than that following the metrazol discharge (2–3 sec.) as well as longer than the pH change with increased blood flow (2–5 sec.). The suppression of metrazol discharge seems, therefore, to be due to anoxia and not to an excess of carbon dioxide or a change in cortical pH.

Summary.—It appears that pH is not a significant factor in the control of the metrazol discharge but that an adequate oxygen supply is essential for its maintenance.*

Discussion

The relative independence of the metrazol discharge from pH changes between 7.1 and 7.5 under the conditions of our experiments does not necessarily imply that cortical facilitation is not affected by pH when tested by electrical stimulation or by the amplitude of spontaneous activity as in the experiments of Dusser de Barenne *et al.* Metrazol may be such a powerful facilitating agent in itself that changes which might appear with pH are "swamped." It seems clear, however, that the facilitating action of metrazol is not mediated by a change in pH nor is it greatly affected by induced changes in alkalinity of fluids bathing the cortical tissue affected. This is at least one type of facilitation mechanism which operates without parallel changes in pH.

The observation that all of the relatively large changes in pH (over 0.1)

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* An experiment on one husky tom cat with "epileptic" tendencies was particularly interesting. A single injection of 0.6 cc. metrazol caused a cyclic violent discharge in the electrogram for over one hour. Curarization was sufficient to eliminate any convulsive movements. pH at the onset was 7.30.

After 18 min. 10 cc. of N/10 HCl was injected i.v. The cortical pH was reduced to 7.20 with no effect on metrazol discharge.

At 24 min. complete cessation of respiration caused a disappearance of cortical activity in 60 sec. even though the pH dropped to only 7.16. After 90 sec. apnea normal respiration was reinstated. Metrazol discharges then reappeared in short bursts after 120 sec. when the pH was still reduced to 7.10.

Hyperventilation was then administered for 4 min. causing a rise in pH to 7.38 at which time the metrazol discharge continued unchanged. Twenty cc. HCl was then injected in the midst of violent cortical activity. This reduced the pH to 7.23 but did *not* stop the metrazol discharge. It was promptly stopped again after 15 sec. apnea (pH 7.15). After a second period of apnea lasting 2 min. normal respiration was reinstated. Large amplitude slow waves at about 2 per sec. appeared in the electrogram after 3 min. normal breathing, pH 7.07. Hyperventilation for 9 min. then caused a reappearance of the metrazol activity and a rise in pH to 7.48. A continuous volley of rapid metrazol discharge then appeared lasting 9 min.

A third injection of HCl (20 cc., making total of 50 cc.) then caused a rapid drop in pH to 6.99 *without* stopping the metrazol discharge although it became less continuous.

Hyperventilation then caused the pH to return to 7.29 at which time the metrazol discharge stopped 80 min. following the single injection of metrazol. A second metrazol injection of 0.6 cc. caused a prompt return of violent cortical discharge. The cat was then given 15 percent CO_2 in oxygen instead of air which caused a rapid fall in pH to below 6.5 again without stopping the metrazol discharge. Metrazol discharge was finally stopped 30 sec. following the beginning of terminal bleeding from the femoral artery.

in an alkaline direction were associated in these experiments with an increase in blood flow and the changes in an acid direction were associated with excessive cortical discharge, inadequately compensated for by increased blood flow, indicates that focal cerebral circulation must be taken into account in all attempts to correlate pH and cortical activity. Since increased cortical discharge of any kind tends to increase the local blood flow, thereby tending to increase the pH of this area, the observed relative alkalinity with facilitation of cortical discharge may be a result, rather than a cause of facilitation. It is highly probable, however, that alkalinity as such may lower the threshold of discharge for cortical neurones as has been shown for peripheral nerve, but that pH is an important mechanism in the physiological control of cortical excitability has yet to be demonstrated.

Conclusions

1. An increase in local cerebral blood flow brought about through the action of adrenalin, metrazol, or by metabolites of increased neuronal activity tends to increase the local pH of the cortex.

2. The vascular dilatation resulting from increased local acidity due to neuronal discharge tends to maintain a constant pH of the cortex under physiological conditions; a delicate homeostatic adjustment mechanism for normal brain function similar to that found in other body tissues.

3. Excessive neuronal activity may exhaust the circulatory adjustment to such an extent as to cause a marked decrease in cortical pH.

4. The onset of a metrazol discharge is characterized first by excessive neuronal activity, then relative acidity followed by relative alkalinity if blood flow is increased. Only acidity results if blood flow is not increased. Increased blood flow with metrazol discharge is regularly observed when the animal is in good condition; failure of increase or a decrease being a sign of deficient reactivity of the cardiovascular system.

5. Apparent small initial "alkaline waves" concomitant with the onset of facilitation in cortical activity may be due to DC voltage components of the cortical electrogram during the violent metrazol discharge. Larger alkaline waves with metrazol discharge were related to overcompensated increase in blood flow.

6. The onset, intensity, duration and form of the metrazol discharge were not appreciably affected by variations in cortical pH between 7.0 and 7.5 induced by hyperventilation, CO_2 breathing, and by the injection of acid or alkaline solutions into the blood stream.

7. Changes in pH accompanying spontaneous cycles of prolonged epileptiform discharges alternating with periods of depressed cortical activity, following a single injection of metrazol, could all be explained upon the basis of the action of "acid" producing neuronal activity and its counteraction by increased blood flow. There was no apparent relationship between the level of pH and the occurrence of prolonged epileptiform discharge of this cyclic type following a moderate initial dose of metrazol.

8. Epileptiform after-discharge of one sigmoid gyrus resulting from nerve impulses conducted from the contralateral cortex subject to electrical stimulation was associated with changes in local cortical pH and blood flow of the discharging area similar in every way to those accompanying epileptiform discharge due to metrazol.

It is concluded that the facilitating action of metrazol, strychnine, and nerve impulses conducted from a distant area of cortex subjected to electrical stimulation is not primarily due to an increase in the local pH of the interstitial fluids of the cortex, because such an increase in pH occurring concomitantly with increased activity of cortical neurones is preceded by an increase in local blood flow. Increased pH seems to be the result of a vascular reaction to increased neuronal activity and does not seem to play an important rôle in the facilitation processes here studied.

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RELATION BETWEEN ELECTRICAL CHANGES DURING NERVE ACTIVITY AND CONCENTRATION OF CHOLINE ESTERASE*

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INTRODUCTION

ELECTRICAL changes during nerve activity occur in milliseconds or even in less than a millisecond. If chemical reactions are closely connected with these changes, the substances involved must appear and disappear with approximately the same rapidity. The difficulty of establishing exact time relations between electrical and chemical changes are therefore obvious. When, in 1933, Kibjakow and Dale and his associates suggested that acetylcholine (ACh) might be the transmitter substance of nerve impulses from pre- to postganglionic fibers (and later from motor nerves to striated muscles) this time factor became of primary importance. For the action of sympathetic and parasympathetic nerves on their effector organs the time factor did not have a comparable significance because cells in these organs react slowly.

No experimental data are yet available which establish the rate of ACh appearance during the period of nerve action. But some indications of the possible rate of removal of ACh from the sites of action have been obtained. ACh is inactivated by the specific enzyme choline esterase. Studies on the concentration and distribution of this enzyme have revealed that at motor end plates and ganglionic synapses as well as at synapses of the central nervous system considerable amounts of ACh can be split in milliseconds. These amounts if liberated would have stimulating action. The experiments, therefore, indicate that the removal of ACh can occur at a rate rapid enough for the assumption that ACh is involved in the transmitter process. The results have been recently reviewed (Nachmansohn, 1940a).

In this paper observations will be described which suggest a relation between electrical changes of nerve activity and the activity of choline esterase.

METHODS

The methods used were the same as described previously (Nachmansohn 1939). The tissues were ground with silicate in a physiological saline solution as homogeneously as possible. The saline solution was artificial sea water according to Pantin with the modification used previously. A fraction of the homogeneous suspension was taken for the determination of the enzyme activity with the manometric method of Barcroft-Warburg.

The determinations on the giant fiber were carried out with the Cartesian diver technique as described by Boell, Needham and Rogers (1939). Here too the tissue was ground with silicate and an aliquot part put into the divers.

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Results

1. *Electromotive force of electric organs and concentration of choline esterase.* The high concentration of choline esterase at muscle end plates induced investigations on the enzyme activity in the electric organs of fishes because these organs are considered as an accumulation of muscle end plates. In view of the great power of the discharge, these organs are suitable for investigating the problem: are electrical phenomena connected with chemical reactions? There is no reason to regard the electrical activity of the elements from which the organ is formed, the electric plates or discs, as extraordinary when compared with a muscle of similar innervation. This has already been emphasized by the physiologists of the nineteenth century (Du Bois-Reymond, 1877, Burdon-Sanderson and Gotch, 1889). The essential difference by which electric organs are distinguished from other excitable structures is the arrangement of the electric plates in series like a Voltaic pile.[1] Hereby the effects of excitation increase in proportion to the number of elements in series.

The powerful electric organs (*Torpedo, Gymnotus*) can discharge 100–200 times per sec. All time relations of a discharge: duration, latency, refractory period, etc., are of the same order of magnitude as those observed in action potentials of ordinary nerves. Therefore, if ACh is involved, the same high concentration of choline esterase must be postulated as in the cases of motor end plates or synapses.

A high concentration of choline esterase was found in the electric organ of *Torpedo marmorata* (Marnay 1937, Nachmansohn and Lederer 1939, Nachmansohn 1940a). 100 g. organ split 200–300 g. ACh in 60 min. These figures appear remarkable for two reasons: (i), the enzyme concentration is of the same order of magnitude as that estimated for motor end plates of frog's sartorius; (ii), the organ has a concentration of a specific enzyme capable of splitting an amount of substrate 2–3 times the total weight of the organ in 60 min., in spite of the fact that electric organs contain about 92 per cent of water and only a little more than 2 per cent of protein. In the electric organ of a *Gymnotus electricus*[2] the enzyme concentration was also high although not as high as in *Torpedo marmorata*. In two species of *Raja undulata* which have a weak electric organ the concentration of choline esterase was relatively low (Nachmansohn 1940a).

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[1] Volta himself detected the analogy between his pile and the electric organ and called the pile an "artificial electric organ" (quoted from Biedermann). There is however an important difference between the Voltaic pile and the electric organ. In the electric organ there is no resting current: if the two ends are connected there is no discharge as in the ordinary pile. The discharge is a voluntary act directed by the central nervous system. This fact induced Berzelius to assume that the discharge is connected with an organic chemical process. In his *Textbook of chemistry* (1817) he describes the electric organs under the title: "Electricity elicited by an organic chemical process" (Nachmansohn 1940b).

[2] The specimen examined died in October 1938 in the Institut Océanographique in Paris and a determination was made on the day following death in the Laboratoire de Physiologie Générale de la Sorbonne.

These observations strongly suggested a "cholinergic" nature of the nervous supply of the electric organ. This assumption was subsequently supported by evidence that ACh is liberated during stimulation and that injection of ACh produces a discharge. Eserine which alone is inactive increases manifold the discharge produced by ACh: 2.5γ of ACh had a

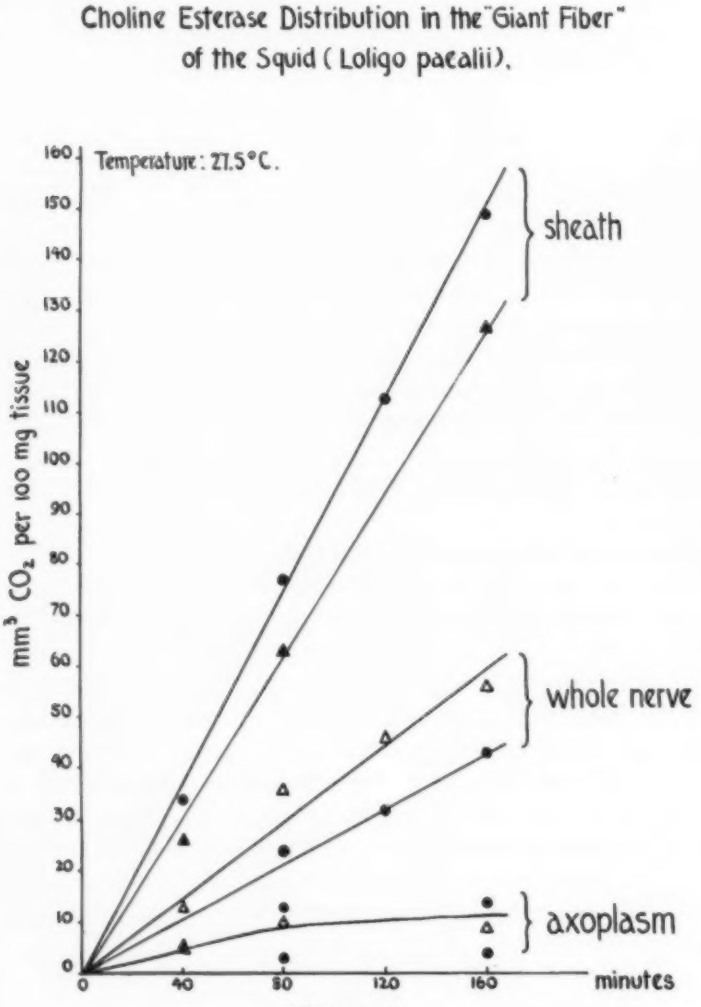


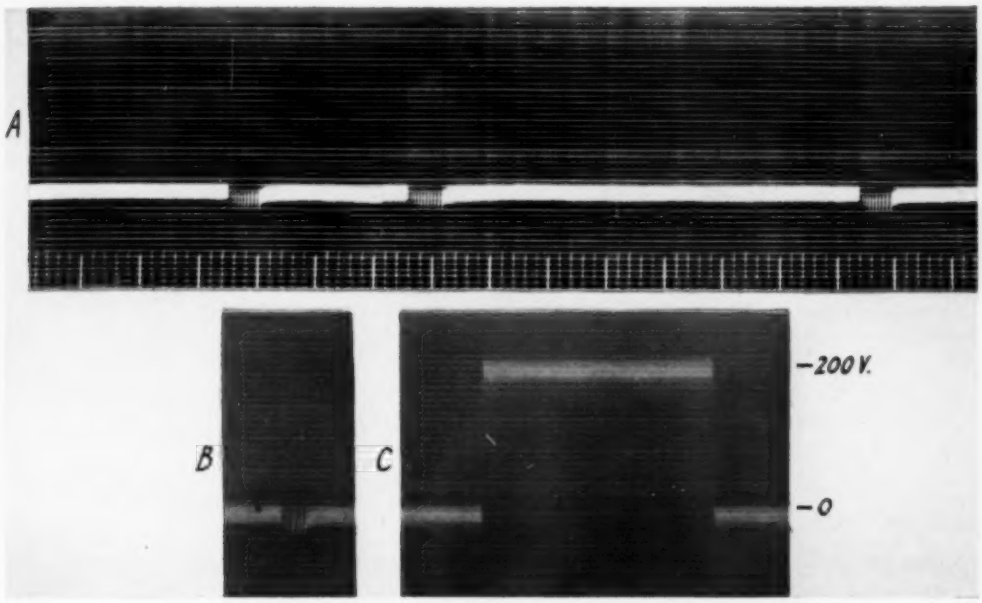
FIGURE 1

stronger effect on the eserinized organ than 100γ before eserinization (Feldberg, Fessard and Nachmansohn, 1940).

If the discharge is closely connected with the appearance of ACh, it should be expected that a quantitative relationship exists between the electromotive force of the discharge, varying from one species to another, and the activity of the specific enzyme which has to remove the active substance during the refractory period. In view of the difference of the arrange-

ment of the electric discs and their number and frequency the best way to find out whether there is a parallelism between the potential differences and the activity of the enzyme appears to be to compare the E.M.F. per cm. and the number of discs per cm. with the concentration of the enzyme. For whatever may be the arrangement of the plates and their total number in series, and whatever may be the E.M.F. of a single plate, the E.M.F. per unit of tissue should be parallel to the concentration of the enzyme if liberation of ACh is directly connected with the potential difference.

The *Torpedo* has the greatest E.M.F. per cm. among the 3 species in-



A & B. Electrical Discharges of Torpedo. String at standard sensitivity. 100,000 ω in series with string. 1 ω in parallel. Peak voltages = A: 170, 155, 175 V. B: 225 V. C. Calibration. Deflection produced by 200 V. applied to large plate electrodes. (R. W. Amberson and D. J. Edwards. See footnote No. 4.)

vestigated. The maximum discharge is about 40–60 V. The extreme values indicated are 30 and 70–80 V. As the height of the columns in medium sized animals is about 1.5–2.5 cm. a cautious estimation would be 10–20 V. per cm., but it is probably nearer to 20 and may reach 30 V per cm. The number of plates in a column is about 400 which would mean an average of 150–250 plates per cm. The maximum discharge of *Gymnotus* according to the data in the literature is between 300 and 800 V. 400–600 V. is probably the most frequent value for the maximum discharge of a medium sized eel. That would be an average of about 6–8 V. per cm. for an 80 cm. organ of an eel of one meter length. But even assuming a larger limit, *e.g.* from 5–10 V. per cm., the E.M.F. per cm. is smaller than in *Torpedo marmorata*. According to the estimations of Ballowitz (1897) the number of discs in the main organ would

be about 60–80 per cm. in medium sized and 100–120 in small animals. In the "bundle of Sachs" the compartments are larger.[3] In the Ray in which there are only about 15 discs per cm. the maximal discharge is only 1–3 V. and 0.5 V. per cm. according to Burdon-Sanderson and Gotch (4).

In Table I the concentration of choline esterase is compared with the estimations for the E.M.F. per cm. and number of plates per cm., in the three species examined: *Gymnotus electricus*, *Torpedo marmorata* and *Raja undulata*. The figures show a definite parallelism. The quantitative relationship may be even more precise if electrical, histological, and chemical studies could be made simultaneously on the same specimen.

In view of the great differences of the maximal discharge it is interesting to compare the maximal discharge with the total amount of ACh which can

Table 1. The relationship between the electromotive force of electric organs per cm. and the concentration of choline esterase.

Species	V per cm.	Plates per cm.	QCh.E.
Raja undulata	0.5	15	3– 10
Gymnotus electricus (larger animals)	5–10	60– 80	90–150
Torpedo marmorata	10–20	150–250	150–300

Table 2. The relationship between maximum discharge of electric organs, number of plates in series and amount of ACh which can be split by the organs in 1 sec.

Species	Maximum discharge in V	Number of plates in series	mg. ACh split by organ in 1 sec.
Raja undulata	1– 3	60– 80	0.5– 1.0
Torpedo marmorata	30– 60	400– 500	50– 100
Gymnotus electricus	300–800	5000–6000	500–1000

be split by an organ in a given period of time. Table II gives such a comparison for the same species which are compared in Table I. Here again there appears a definite parallelism. Such a parallelism is however less conclusive, for it is possible to imagine that in an organ the number of columns in parallel reaches very high values without a similar great number of plates in series. In that case the total amount of ACh which can be split in a given unit of time may be higher than the corresponding E.M.F.; only the amperage and power output would be higher.

We had the opportunity of examining 3 specimens of the large *Torpedo* (*T. occidentalis Storer*) which occasionally occurs in the water around Cape

[3] These estimations are based on the assumption that E.M.F. per cm. and number of plates per cm. and enzyme concentration are rather uniform throughout the organ. More recent and detailed investigations however give a different picture (Coates, Cox and Nachmansohn).

Cod. The electric organs in this species are several times as large as in the *Torpedo marmorata*. There are no indications in the literature concerning the number of plates in series. The shock is described as being of a great power and only a little less strong than that of *Gymnotus* (Fritsch 1883, Rosenberg 1928). The E.M.F. of the discharge was recorded with a string galvanometer by W. R. Amberson and D. J. Edwards.[4] Record A shows peak voltages of 170, 155 and 175 V., record B one of 225 V. Record C is a calibration record which shows the extent of galvanometer deflection caused by a voltage of 200 impressed upon the large metal plates which the authors used to press against the upper and lower surfaces of the animal. In most of the discharges

Table 3. Concentration of choline esterase in electric organs of Torpedo occidentalis, Storer

No.	Wt. of animal kg.	Length cm.	Width cm.	Ht. of columns cm.	Wt. of 2 elec. organs kg.	mg. wt. taken for determ. mg.	QCh.E.	TCh.E
1	13.5	—	—	2.8	2.5	12.0 43.5	*284.0* *290.0* *271.0*	7.0
2	39.2	123	90	7.6	9.0	22.0	*79.0* *73.0* *80.5*	7.0
3	9.0	80	52	3.0	2.0	27.0	*226.0* *227.0* *211.0*	4.0

TCh.E. = total amount of ACh in kg., which can be split by the 2 organs in 60 min.

the voltage was between 150 and 250 V. The authors feel that the peak voltages are actually higher than the values indicated by these records. The string galvanometer used was of a rather obsolete type and can hardly be expected to register the voltage accurately for such transient electrical discharges. In any case the voltages are no less than the records indicate.

The values for the concentration of choline esterase in the three specimens examined are high in the two smaller animals; in fact they are as high as in *T. marmorata* (Table III). This coincides with the observation that the discharge is much more powerful in the *T. occidentalis* than in the *T. marmorata*. As the electric organs in the former are several times as large as in the latter it should be expected that at the same enzyme concentration the discharge is more powerful in the large organs if E.M.F. and enzyme activity are correlated. In the giant animal the enzyme concentration is

[4] Unpublished observations. The authors permitted us to mention their results and to reproduce some of their records in this paper. It is a great pleasure to express our gratitude for their kindness.

about one-third of that in the two smaller animals. This again is not surprising. The number of plates does not vary considerably from one individual to the other in a given species. Therefore as the height of the columns in the giant animal is 2–3 times as high as in the two others the concentration of choline esterase should be proportionally lower.

It has previously been shown (Feldberg, Fessard and Nachmansohn) that in the *T. marmorata*, the electric lobes and the nerves which come from these lobes and innervate the electric organs have a considerably higher enzyme concentration than the rest of the central nervous system. Therefore the distribution of choline esterase was determined in the central nervous system of a giant *Torpedo*. Table IV gives the data on some parts of the central nervous system. Although the electric lobes and the nerves innervating the electric organs have a relatively higher concentration than

Table 4. Concentration of choline esterase in some parts of the C.N.S. of the giant Torpedo occidentalis, Storer. Weight 34.5 kg. Length 132 cm.

Part of the C.N.S.	Wt. mg.	mg. wt. taken for determ.	QCh.E	TCh.E.
Cerebral hemisphere	370	=	*1.4* *1.3*	5.3
Cerebellum		224.5	*1.4* *1.3*	3.0
Hypophysis	52	=	*0.98*	0.5
Spinal cord		125.6	*2.1*	
Optical tract		26.0	*2.0*	
2 electric lobes	2308	37.0	*4.2*	97.0
Electric nerve near el. lobe		117.0	*5.0*	
Electric nerve near el. lobe		75.0	*5.1*	

the other parts of the central nervous system, the difference is not as great as that in *T. marmorata* and does not appear significant. The evalues do not lend support to the assumption that the nerves of the electric organs are distinguished by a higher concentration than ordinary nerves. Only the amount of nervous tissue is important and therefore the total amount of enzyme in the electric lobes as indicated by the TCh.E. is relatively high.

The enzyme concentration was also determined in specimens of two more species of Rays, *Raja erinacea* and *Raja eglanteria*. The figures obtained are given in Table V. The concentration is a little higher than that found in *Raja undulata*, but still of the same order of magnitude and low compared with that of the powerful electric organs.

II. *Localization of choline esterase in nerve cell.* The relationship between E.M.F. of the discharge in electric organs and the activity of choline esterase

leads to the question: is this relationship limited to nerve endings, either as the final event in the discharge of the electric organs or as part of the transmission process at synapses and motor end plates? Or are the potential differences which occur if nerve impulses travel down a nerve fiber alike and therefore also connected with ACh metabolism? Gasser and Erlanger (1939) in recently scrutinizing the problem came to the definite conclusion based on the electrical phenomena that conduction along fibers and across synapses is essentially the same process, as conceived previously by Eccles and Sherrington, and that the difference is only a quantitative one.

Liberation of ACh was generally considered as a process specifically limited to nerve endings and the quaternary base was therefore referred to as

Table 5. Concentration of choline esterase in electric organs of Ray.

No.	Species	Wt. animal gr.	Wt. el. organ taken for determin. mg.	QCh.E.
1	*Raja erinacea*	1000	71.0 97.0	*17.5* *18.3*
2	*Raja erinacea*	1750	91.4 104.4	*15.1* *13.8*
3	*Raja eglanteria*	1850	61.5 82.0	*13.8* *14.0*
4	*Raja eglanteria*	2250	37.0 75.6	*12.6* *12.8*
5	*Raja eglanteria*	1200	31.5 20.2	*15.9* *19.2*

"synaptic transmitter." When, in 1938, Lorente de Nó found that ACh was liberated not only at synapses but also at fibers, his results were strongly criticized by Dale and his associates and a controversy arose over this problem (8, 14, 20, 21).

It has been emphasized on many occasions since 1938 that choline esterase is highly concentrated in all nerve fibers although it rises still more at regions where synapses and motor end plates exist. (Nachmansohn, 1938a and b, 1939). This was particularly obvious in non myelinated fibers, *e.g.* in the abdominal chain of lobsters where the QCh.E. values in the fibers were as high as 5.0–15.0, and rise at the points where the synapses are located to values of 18.0–30.0. There was a similar relationship in the distribution of the enzyme in the sympathetic chain of mammals. The conclusion was drawn from these observations that ACh metabolism in fibers and at synapses differs only quantitatively (25, 26).

Experiments carried out on the superior cervical ganglion of cats after section of preganglionic fibers suggest that the difference in enzyme con-

cencentration between fiber and synapses may be connected with the difference of surface extension (Couteaux and Nachmansohn, 1939, 1940). The concentration of choline esterase in the ganglion decreases during the first ten days after section of preganglionic fibers by about 60 per cent and remains then stable for many weeks. As the decrease is parallel to the disappearance of the preganglionic fibers, it is reasonable to assume that the enzyme which disappeared was located *inside* the fibers, whereas the remaining enzyme was located *outside* the fibers. Under this assumption the figures obtained indicate that the enzyme concentration of the preganglionic fibers inside the ganglion is several times higher than the concentration in the same fibers before they enter the ganglion. These observations lead to the

Table 6. Distribution of choline esterase in the giant fiber of the squid (Loligo paealii) Temperature: 27.5°C.

No.	Kind of tissue	Mg. fresh tissue ground	Mg. fresh tissue per diver	Duration of experim. min.	Output of $CO_2 \times 10^3$ mm.^{3}	QCh.E. (average)
1	Whole axon	2.6	0.078 0.078	185	141 118	*0.438*
	Sheath	0.55	0.017	185	69	*1.130*
	Axoplasm	2.6	0.078	185	12	*0.040*
2	Whole axon	3.3	0.123 0.123	160	53 69	*0.150*
	Sheath	2.4	0.09 0.09	160	135 115	*0.420*
	Axoplasm	5.5	0.20 0.20 0.20	160	8 28 18	*0.027*

conclusion that the strong increase of enzyme concentration in the preganglionic fibers towards their endings may be connected with the increase of surface occurring inside the ganglion by the extensive endarborization of the preganglionic fibers. They suggest that the enzyme may be higher concentrated at or near the surface and that the increase of surface explains the high concentration at synapses.

Bioelectric potentials are, it is generally believed, surface phenomena. Hodgkin and Huxley were recently able to prove this conception by measuring the potential difference between the inside and the outside of the giant axon of Squids (1939). The exact site of the membrane and the nature of the alteration which occurs there is not yet elucidated. Höber *et al.* (1939) studying the depolarizing effect of numerous organic and inorganic electrolytes and non-electrolytes recently discussed the different possibilities and aspects of the problem.

Table 7. Concentration of choline esterase in nervous tissue of Squids.

a. In fibers				b. In ganglia			
No.	Tissue	Wt. taken for det. mg.	QCh.E.	No.	Tissue	Wt.[1] mg.	QCh.E.
1	Giant fiber	10.0	*0.280*	1	Stellate ganglion	35.4[2]	*11.5*
2	Giant fiber	8.5	*0.214*	2	Stellate ganglion	24.4	*8.0*
3	Giant fiber	12.5	*0.210*	3	Stellate ganglion	26.2	*9.6*
4	Giant fiber	11.5	*0.170*	4	Stellate ganglion	22.0	*9.6*
5	Whole trunk, containing giant fiber	21.6	*0.444*	5	Head ganglion	167.0	*425.0* *465.0*
6	Whole traunk, containing giant fiber	22.6	*0.750*	6	Head ganglion	198.0	*307.0* *302.0*
7	Whole trunk, containing giant fiber	29.6	*0.544*	7	Head ganglion	175.0	*370.0* *363.0*
8	Finnerve	28.2	*0.500*	8	Head ganglion	167.0	*270.0* *280.0*
9	Finnerve	27.2	*0.485*				
10	Finnerve	33.6	*0.320*				
11	Finnerve	23.6	*0.416*				
12	Finnerve	26.0	*0.510*				

[1] The weight indicates the total weight of the ganglion. The total amount was ground and an aliquot part taken for determinations.

[2] Two ganglia.

Obviously if choline esterase is concentrated at or near the surface of nerve fibers, as the experiments on the superior cervical gangion suggest, it would strongly support the assumption that ACh is one of the substances involved in the electrochemical changes occurring at the surface during nerve activity.

Direct evidence is offered for this assumption with experiments on the giant fiber of the Squid.[5] Determinations of choline esterase carried out with the diver technique as developed by Boell, Needham and Rogers, show that practically all the enzyme is localized in the sheath. The esterase activity of the axoplasm is negligible. Table VI gives the data obtained in two experiments. Experiment 2 was carried out with two and in the case of axoplasm with three parallel determinations. Figure 1 demonstrates the difference of the rate of hydrolysis in sheath, whole fiber and axoplasm respectively. 80–90 per cent of the sheath is connective tissue. The values for the sheath have

[5] These experiments were made in collaboration with Dr. E. J. Boell of the Osborn Zoological Laboratory, Yale University. A preliminary report was given in *Science*, 1940, *92*: 513–514.

therefore to be multiplied 5–10 times. But even then the values are minimum values: the enzyme concentration may be actually much higher than the activity per unit of tissue weight indicates, because it may be localized in a small fraction of the remaining volume.

The esterase concentration in the total giant fiber is intermediate between that for the sheath and that for the axoplasm. The values for the giant fiber with the ordinary Barcroft-Warburg method are about the same as those with the diver technique (see Table VII). Determinations on the total trunk containing the giant fiber reveal that the QCh.E. is higher than in the giant fiber itself. This is to be expected, for if the enzyme is concentrated at or near the surface, fibers with a smaller diameter should have a higher concentration of enzyme per unit of weight. As the fibers running in the same trunk have a smaller diameter than the giant fiber, the increase of QCh.E. is in agreement with that assumption. The trunk containing the giant fiber is composed of different types of fibers. Therefore the QCh.E. of the fin nerve which contains only motor nerve fibers has also been determined in order to compare functionally identical nerve fibers varying only in diameter. The values here too are definitely higher than in the giant fiber. The values in the stellate ganglion are about twenty times higher than in the giant fiber, the QCh.E. being about 8.0–11.0. This difference is of the same order of magnitude as that found between preganglionic fibers and the superior cervical ganglion of cats.

A particularly high value was found in the head ganglion exceeding all others so far, even those in the electric organ of *Torpedo*. It must be kept in mind however, that the protein content is about ten times higher in the head ganglion than in electric organs: it varied between 18 and 23 per cent

Discussion

Two main facts result from the observations described: (i), the parallelism between E.M.F. and activity of choline esterase in electric organs indicates a quantitative relationship between potential differences of nerve activity and ACh metabolism. (ii) The enzyme is highly concentrated everywhere at or near the surface of the nerve cell suggesting that ACh metabolism occurs not only at synapses but everywhere at the surface of nerve cells, and is everywhere connected with the potential differences during nerve activity. The difference is only a quantitative one, correlated to the increase of surface at synapses. This again is in agreement with the observations on electrical changes from which Gasser and Erlanger came to the conclusion that there exists only a quantitative difference between conduction along fibers and across synapses.

Thus the battle cries of the last years: "electrical transmission" and "chemical transmission" lose their significance, because, in view of the results reported here, it is difficult to maintain the concept that ACh has a "transmitter" function specifically limited to nerve endings. The experiments suggest—in modification of the original theories of Loewi and Dale—

that ACh metabolism is intrinsically connected with the electrical changes of nerve activity which occur at the surface of nerve cells.

The objection may be raised that the conclusions are based on the presence of the enzyme and its concentration and not on determinations of the amounts of the ester actually metabolized. Choline esterase is a specific enzyme (Easson and Stedman 1937, Glick 1938, 1939). If a specific enzyme is highly concentrated in a cell, one may assume that the substrate is metabolized in such cells and that a relation exists between the concentration of the enzyme and the rate of the metabolism of its substrate. For such an assumption convincing evidence has been brought forward by the use of isotopes in the study of metabolism. As Schoenheimer and Rittenberg (1940) state in their review, the results with isotopes do not support the assumption that the tissue enzymes lie dormant during life, as some investigators believe: "The experiments indicate that all the actions for which specific enzymes and substrates exist in the animal are carried out continuously." This statement is particularly pertinent in view of the fact that the experiments with isotopes are carried out on intact animals.

At present it is difficult to go beyond the conclusion that ACh metabolism is closely connected with electrical changes occurring during nerve activity at the surface of neurons. A more precise formulation would require a knowledge of the time course of the appearance of ACh. This appearance may be the first, second or even the third step in a chain of events. It is hard to conceive that a biological process such as conduction or transmission of nerve impulses should be connected with a single chemical reaction.

Summary

1. If in the electric organs of Ray, *Torpedo marmorata* and *Gymnotus electricus* voltage and number of plates per cm. are compared with the concentration of choline esterase, a close parallelism appears between E.M.F. and activity of the enzyme.

2. The enzyme concentration in the electric organ of the giant *Torpedo occidentalis Storer* is as high as in the electric organ of *T. marmorata*. This coincides with the observation that in the larger organs of the former species the E.M.F. is several times greater than in the small organs of the latter species.

3. No significant difference was found between enzyme concentration in electric lobes and nerves supplying the electric organs and that in other parts of the central nervous system in *T. occidentalis*.

4. Determinations of choline esterase carried out separately with sheath and axoplasm of the giant fiber of Squids (*Loligo paealii*) show that practically all the enzyme is localized in the sheath: the esterase activity of the axoplasm is negligible. This is considered as direct evidence for the previous assumption that the enzyme is concentrated everywhere at or near the surface of the nerve cell and that the rise of concentration at synapses is connected with the increase of surface.

5. In the head ganglion of the Squid a concentration of choline esterase has been found which is higher than in any other tissue examined thus far.

6. It is suggested that the potential differences observed during nerve activity may be closely connected with the metabolism of ACh everywhere at or near the surface of the nerve cell, the metabolism being only quantitatively more important at synapses.

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NATURE OF THE "ENDPLATE POTENTIAL" IN CURARIZED MUSCLE

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THE PROBLEM of neuro-muscular transmission may be divided into four successive stages: (i) the process whereby the nerve impulse gives rise to the transmitting agents; (ii) the nature of the transmitter, and the mode of its action and subsequent inactivation; (iii) the local changes set up at the junctional region of the muscle fibre; (iv) the initiation of a propagated muscle impulse by these local changes.

The present investigation deals with the later phases of the transmission process (problems iii and iv above). During a state of neuromuscular block by curare, local action potentials can be recorded at the junctional region of the muscle. Their properties and their relation to the propagating impulse have been studied in detail in this and following papers. The evidence so obtained concerns primarily the initiation of the muscle impulse, but indirectly throws some light also on the transmitter problem (No. ii above).

METHODS

For an electrical study of neuro-muscular transmission, it would be desirable to work on a completely isolated nerve-muscle fibre. A strip of parallel muscle fibres, however, with a sharply localized "focus" of motor nerve endings would be, for many purposes, quite a satisfactory substitute. As has previously been shown (Katz and Kuffler, 1941), the frog's sartorius provides frequently a very suitable object, most of its fibres being innervated at two or several discrete "end-plate" zones. The innervated strip preparation of the cat's soleus (Eccles and O'Connor, 1939) has also been used.

The isolated frog's sartorius was curarized by soaking for 20–30 min. in Ringer's solution containing curarine in concentrations from 1 μmol. per l. (3.3×10^{-7}) upwards. We are indebted to Dr. H. King of the National Institute for Medical Research, Hampstead, for kindly supplying us with purified curarine chloride. Curarization of the cat's soleus was effected by intravenous injection of solutions of highly concentrated curare (Merck). 2 mg. per kg. usually proved sufficient to give complete paralysis for some minutes.

The apparatus for stimulation of the nerve or muscle and electric recording from the endplate region has been described elsewhere (Eccles and O'Connor, 1939; Katz and Kuffler, 1941); for fast recording, an approximately linear sweep controlled by the discharge of a gas-filled triode through a variable resistance-capacity network was employed. To calibrate the amplifier and time base, the Lucas pendulum was used to supply rectangular current pulses. A simple test for the linearity of the time base was provided by recording a series of damped oscillations which were obtained by connecting a condenser across the secondary of the stimulating induction coil. The amplifier response to a rectangular voltage input reached 90 per cent of the full deflection in 0.1 msec. and fell to half in about 0.2 sec. When necessary the rate of decline was reduced by choosing larger coupling condensers. In most cases no correction was required for amplifier distortion.

RESULTS

A. SPATIAL DISTRIBUTION OF THE ENDPLATE POTENTIAL IN CURARIZED MUSCLE

When a muscle is sufficiently poisoned by curarine, a nerve volley fails to set up a propagated muscle spike and the accompanying twitch, but it

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still gives rise to a small negative potential change at the regions of the neuro-muscular junctions. This local depolarization (the "end-plate potential" of Göpfert and Schaefer, 1938, and Eccles and O'Connor, 1939) (Fig. 1 and 2) can easily be distinguished from the ordinary muscle spike, (i) be-

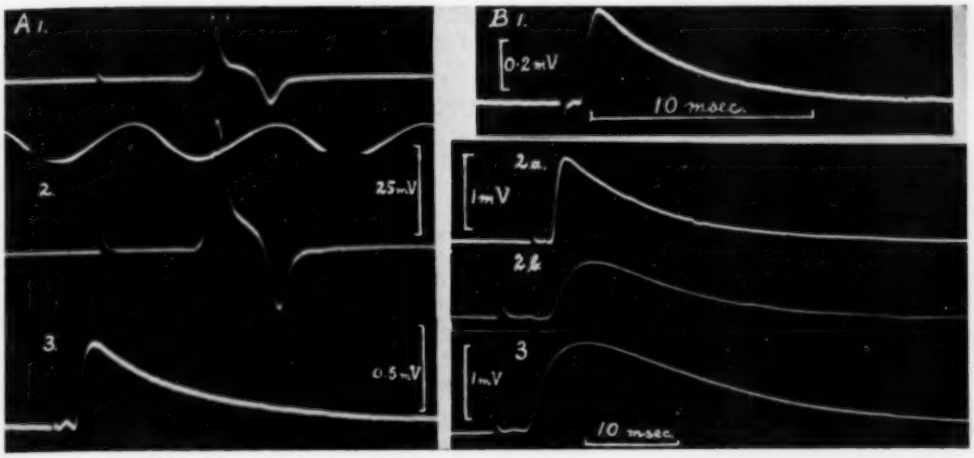

FIG. 1. "Endplate potentials" (e.p.p.'s) in curarized muscles. *A1* and *2:* Diphasic spike potentials of sartorius muscle at 25°C., due to direct stimulation; 1 half-maximal, 2 maximal; distance between leads 9.5 mm. *3:* E.P.P., due to nerve stimulation. Time signal: 1 d.v. = 10 msec. Note: The small wave preceding the e.p.p. is due to the action potential of the intramuscular nerves (see also Fig. 4). *B1:* E.P.P. in cat's soleus muscle (at 38°C.). *B2:* E.P.P. in frog's sartorius; *a*, at 23°C.; *b*, at 10°C. *B3:* Another frog's sartorius, at 13.5°C.

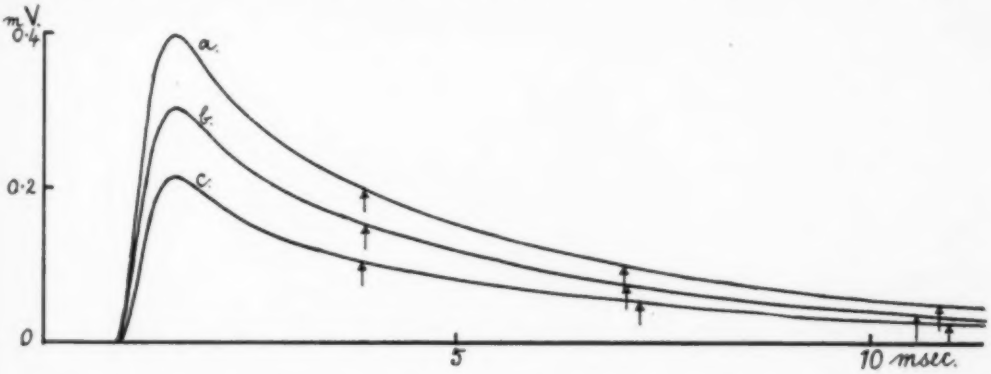

FIG. 2. Cat's soleus. E.p.p.'s with arrows showing points for decay to $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ of the summit height. (a) single nerve volley; (b) diminished response to second nerve volley at 24 msec. after first; (c) response to single volley diminished to nearly one half by further curarization. There is no significant difference in the three curves, and their successive half times progressively increase, being approximately 2.3, 3.3 and 3.7 msec.

cause it is not conducted along the fibres and (ii) because of its slower decay. It has been shown previously (Eccles and O'Connor, 1939; Katz and Kuffler, 1941) that the "endplate zones" can be located as the regions of minimum spike latency in the normal muscle, *e.g.* Fig. 3 gives evidence of several

such regions at which motor endings are accumulated. When the muscle is curarized, end-plate potentials (e.p.p.) are recorded at these regions and are often observed to decrement sharply at either side, becoming imperceptible a few millimetres away. In Fig. 4, for instance, there is a rapid diminution in size as the recording electrode is moved in either direction from the 11 mm. position; at the same time the e.p.p. waxes and wanes progressively more

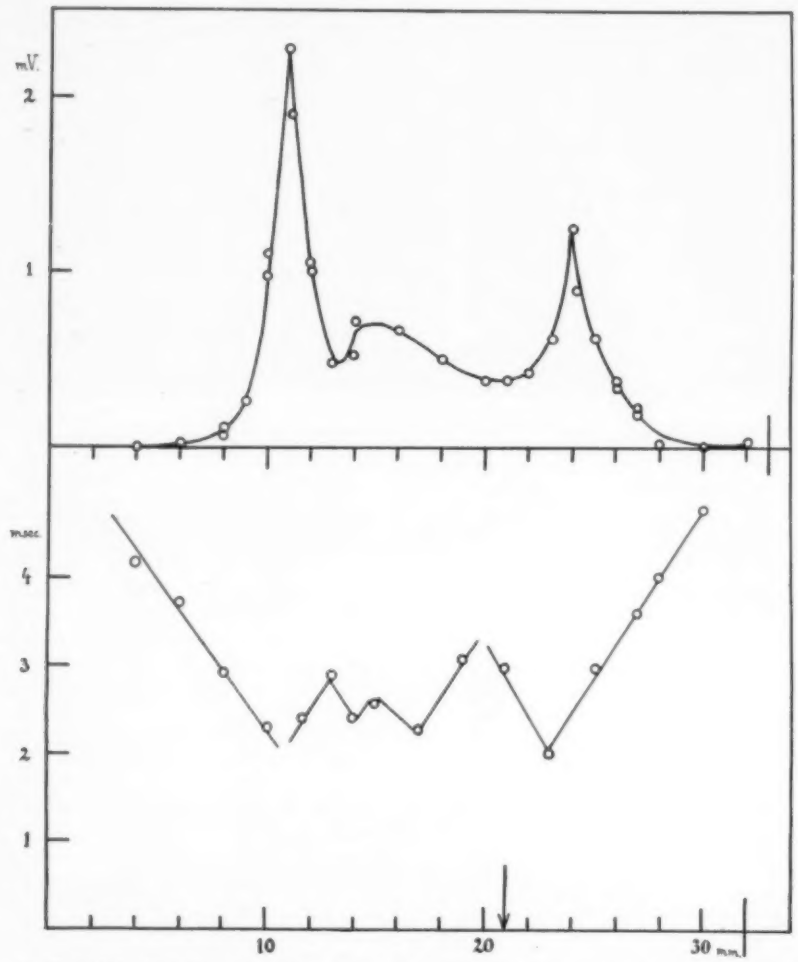

FIG. 3. Spatial distribution of e.p.p. in curarized frog's sartorius. Temp. 27.5°C. *Upper part:* completely curarized. Ordinates: Size of e.p.p. Abscissae: Position of earth lead, in mm. distance from pelvic end. Grid lead at pelvic end. Resting length 33 mm. *Lower part:* same muscle normally. Ordinates: Latency of muscle spike due to a nerve volley (shock-peak interval). Abscissae: as before. Resting length 32 mm. Position of nerve entry marked by arrow. Note the large e.p.p.'s at the regions of spike origin (at 11 mm. and 23–24 mm.).

slowly. In four preparations containing a sharply localized endplate focus the peak of the e.p.p. was found to decay to half in 0.5–0.9 mm. (fall to $1/e$ in 0.7 to 1.25 mm).

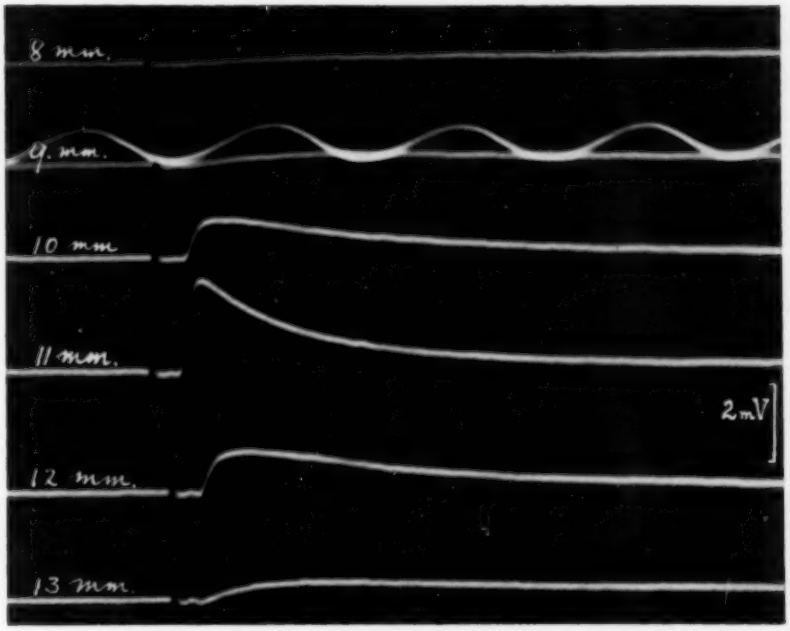

FIG. 4. Spatial spread of e.p.p. Same muscle as in Figs. 3, 5, 6. Records taken with grid lead at pelvic end and earth lead at a distance of 8–13 mm., as shown. Time signal: 1 d.v. = 10 msec. Note appearance of small nerve spike preceding the e.p.p., as electrode is moved towards nerve entry.

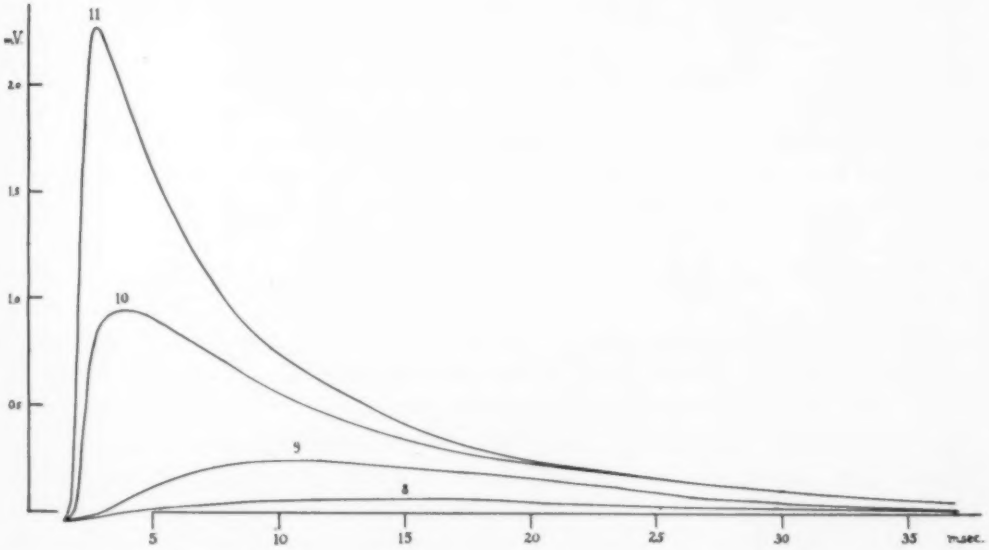

FIG. 5. Spatial decrement and accompanying change in time course of e.p.p. Records of Fig. 4 plotted and superimposed. Position of earth lead, successively from above: 11, 10, 9, 8 mm. distance from pelvic end. Abscissae: Time after nerve stimulus.

The characteristic spread of the e.p.p. in the vicinity of the motor nerve endings is of great interest. Its exponential decrement along the muscle and the associated slowing of its time course resembles the spread of electrotonic currents in nerve and muscle, or—more generally speaking—the spread of electric charge along a leaky capacitative cable (Cremer, 1909; Rushton,

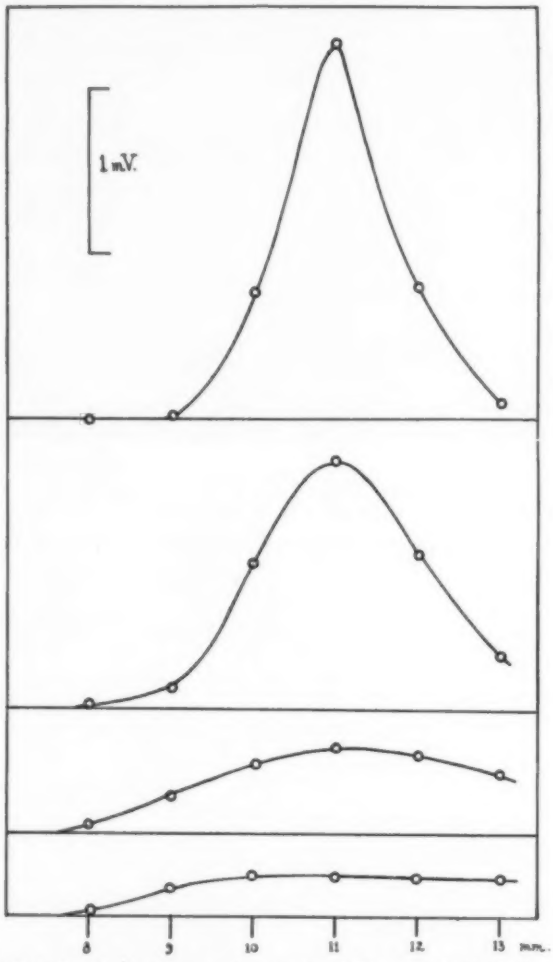

FIG. 6. Spatial distribution of e.p.p. (records of Fig. 4) plotted at successive time intervals. Time after nerve stimulus, successively from above: 2.8 msec.; 5.4 msec.; 13.3 msec.; 20 msec. Abscissae: Distance from pelvic end.

1934). An electric potential change set up at any given point of a nerve or muscle fibre causes the flow of local currents which spread a certain distance along the fibre, depending upon the resistance of the fibre and especially upon the transverse impedance of its surface membrane. In medullated nerve, the electrotonic potential spreads more extensively than in non-medullated nerve or muscle (the distances for $\frac{1}{2}$ decay are 2–3 mm. in frog's medullated nerve (Rushton, 1934; Bogue and Rosenberg, 1934;

Hodgkin, 1937), while only 0.5 mm. in crab's nerve (Hodgkin, 1938), and about 1 mm. in the frog's sartorius (Schaefer, Schölmerich and Haass, 1938). Since the surface membrane has a large distributed capacity (Cole and Curtis, 1936), the electrotonic potential does not rise instantly but only gradually, depending upon the rate of charge of the local membrane capacities. Thus, the greater the distance from the endplate focus, the slower is the rise of the recorded potential wave (Fig. 4 and 5) due to the delaying action of the intermediate membrane capacities. If the spatial distribution of the e.p.p. is plotted at successive intervals after the arrival of the nerve volley (Fig. 6), it is found that the e.p.p. continues to spread laterally even during its decay, in exact accordance with theory (Cremer, 1909).

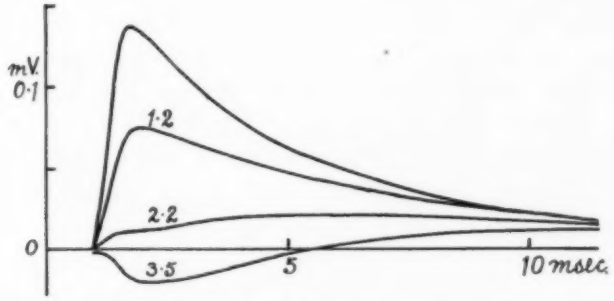

FIG. 7. Endplate potentials set up by a single nerve volley at various points along an innervated soleus strip of a cat. The largest potential is recorded at the endplate focus and the others at the indicated distances in mm. from the focus.

It might be argued that the spatial decrement of the e.p.p. is due to a scattered distribution of motor endings rather than to an extrinsic current spread from an adjacent focus of depolarization. In that case, however, the time course of the e.p.p. should be invariable and not exhibit the characteristic slowing associated with electrotonic spread. In the example of Fig. 4 a lateral scatter of motor endings more than 1 mm. away from the focal zone (at 11 mm.) cannot play an important part: at 9 mm. for instance, the e.p.p. reaches at its crest 12 per cent of the maximum peak (at 11 mm.), but its rate of rise is less than 2 per cent of that at the "focal region." It appears, therefore, that only a small fraction of the e.p.p. at 9 mm. can be due to the presence of scattered junctions at this point, and that at least 85 per cent of it is electrotonically transmitted from the focal zone 1–2 mm. away. Frequently, however, the endplates are not so sharply localized, and then, of course, the spatial potential gradient is made up in an unknown proportion by both factors: (i) the local density of endplates, and (ii) the electrotonic spread of depolarization from adjacent endplates.

With the soleus strip preparation of the cat the e.p.p. also becomes smaller and slower as the electrode is moved away from the endplate focus. At a distance of a few millimetres, however, the negative potential change is preceded by a positive initial wave (Fig. 7). This effect is due, not to an es-

sential difference between cat and frog muscle, but to the different conditions of electric recording in the two cases. As has been shown by Bishop (1937) and Bishop and Gilson (1929), an extensive fluid or tissue shunt, such as the inactive remainder of the cat's soleus muscle, distorts the local circuits in the vicinity of a depolarized region of the muscle surface in such a way as to make adjacent regions relatively positive to the more distal (cf. also Eccles and O'Connor 1939, p. 49). The effect can be imitated on the frog's sartorius by embedding it in a saline bath. This distortion precludes, of course, any accurate estimation of the spatial electrotonic decrement for the cat's soleus, an approximate value being half decay per millimetre.

B. SIZE AND TIME COURSE OF THE ENDPLATE POTENTIAL IN RELATION TO THE MUSCLE SPIKE

It is clear from the preceding section that the time course of the e.p.p. depends to some extent upon the scatter of nerve endings within any particular junctional zone: for example, an endplate focus spread over 2 mm. will give a slightly slower e.p.p. than a focus restricted to 0.5–1 mm. of muscle length. In Table 1 the size and time course of the e.p.p. is compared with that of the normal propagating spike, both being recorded from the centre of a junctional region.

Table 1. End plate potential and normal muscle spike

(*cf.* Fig. 1 and 2) For typical records of spike potentials at the junctional region, see Eccles and O'Connor, 1939 (cat's soleus) and Katz and Kuffler, 1941 (frog's sartorius).

"Latency" (Maximum interval between arrival of nerve spike at junction and beginning of e.p.p. or spike respectively). msec.	*Rising phase* msec.	*Fall to 1/e* msec.	*Size*		
1.1–1.5	2.3	11	2–4 mV	e.p.p. (critically curarized).	Frog's sartorius at 20° C.
1.1–1.5	1.6	1.5	40–50 mV	spike	
0.6	0.8	4.5	5 p. c.	e.p.p.	Cat's soleus.
0.7	0.6	0.6	100 p. c.	spike.	

(i) *Latency.* The delay between the nerve stimulus and the start of the potential change at the endplate zone is but little altered by curarization. A large part of this delay is due to nerve conduction; as can be shown either by calculation or directly by high-amplification records which reveal the action potential of the intra-muscular nerves in the vicinity of the endplates (Fig. 1, 4). There remains a latency between the beginnings of this small nerve spike and of the muscle spike, or e.p.p. respectively, amounting

to 1.35 msec. (1.1–1.5 msec.) in the frog's sartorius at 20°C. and 0.6–0.7 msec. in the cat's soleus (Table 1). This delay hardly exceeds the duration of the nerve spike, and a fraction of it must again be due to conduction along the terminal nerve branches. It is affected by temperature changes to about the same extent as the duration or conduction rate of nerve or muscle impulses (see Table 3 below).

(ii) *Size.* The recorded size of e.p.p. and spike depends, though of course to a different extent, upon the distribution and local density of end-plates. The ratio of e.p.p. to spike must be greater the more strictly localized the end-plate zone, and greatest in a single isolated fibre. In 6 frog sartorii with relatively sharp endplate foci, the e.p.p. in critically curarized muscle (*i.e.* when abolition of spikes and associated twitches was just complete) reached 5–8 per cent of the normal spike (2–4 mV out of 30–50 mV); in 12 experiments on the cat the ratio was 3–6 per cent.

If the dose of curarine is increased beyond that required for complete paralysis, the e.p.p. becomes progressively smaller without, however, altering its time course (Fig. 2). Table 2 shows the relation between size of e.p.p. and curarine concentration.

Table 2. Relation between size of e.p.p. and dose of curarine

Frog's sartorius, at 16°C.					
Concentration of curarine, in μmol./l.	2	3	4	5	6
Size of e.p.p. in mV.	2.7*	1.2	0.75	0.5	0.34

* Spikes are still present in some fibres; peak of e.p.p. obtained by extrapolation of the rising phase, which has the same initial time course as with larger doses of curarine.

(iii) *Time course.* The e.p.p. is distinguished from the spike by a much slower decay. Its descending phase is approximately exponential, with a time constant of about 10 msec. at 20°C. for the frog's sartorius and 5 msec. for the cat's soleus. It has been pointed out by Schaefer and Haass (1939), (cf. also Katz, 1938) that the decay of the e.p.p. involves a time factor which is nearly the same as the time constant of the electrotonic potential (Schaefer, Schölmerich and Haass, 1938) or of the α-excitability of skeletal muscle (Rushton, 1930). In other words, the decline of the e.p.p. has about the same time course as that of an electric potential change in the muscle produced by an extrinsic current pulse. This relation is important because it suggests that the descending phase of the e.p.p. represents merely the passive decay of an electric change previously set up at the muscle membrane, in the absence of any further disturbing cause, just as would occur *after the withdrawal* of an applied cathodal current pulse (*cf.* Hill, 1936; Katz, 1939).

There are, however, both theoretical and experimental reasons (Cremer, 1909; Katz, 1939) for believing that the decay of the electrotonic potential in muscle does not follow a truly exponential time course, but is a function of the spatial and temporal distribution of

the applied current, *e.g.* the longer the current pulse, the slower should be the decline of the electrotonic potential. According to the electrotonic theory, successive half-times of decay of the e.p.p. should lengthen progressively. This is frequently observed (see *e.g.* Fig. 2), especially when the endplate focus is sharply localized. It may be that the strictly exponential decay found in many other preparations is really a distortion due to electrotonic potential spread from adjacent endplates. As a whole, therefore, the similarity of the time courses of the decay of e.p.p. and catelectrotonic potential, while suggestive of a similar mechanism, must be regarded with some caution.

C. THE EFFECT OF TEMPERATURE ON THE E.P.P.

In a series of experiments on the frog's sartorius, the effect of temperature on the time course of the e.p.p. was studied. In each experiment observations were made at two temperatures differing by 10 to 15°C., and ranging between 7 to 26°C., and were completed by returning to the original or an intermediate temperature. The results are illustrated in Fig. 8 and summa-

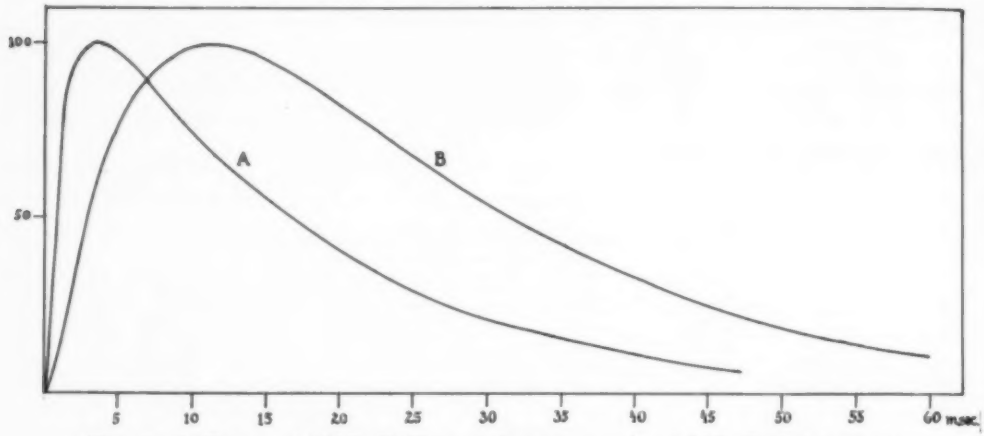

FIG. 8. *A*. E.p.p. at 19°C. *B*. E.p.p. of same muscle at 9°C. Scaled to same maximum. (The e.p.p. was 40% smaller at 9°C. than at 19°C.)

rized in Table 3. They reveal that the time course of decay of the e.p.p. is but little affected by temperature change ($Q_{10} = 1.3$), while the duration of the rising phase is lengthened nearly three times by a 10°C. lowering of temperature. It is clear that different processes underlie the building up and the decay of the e.p.p. It has been suggested above that the decay is mainly due to a passive dissipation of electrotonic charge along and across the muscle membrane. This view is supported by the low temperature coefficient (cf. Bogue and Rosenberg, 1934; Gasser, in Erlanger and Gasser, 1937). On the other hand, the building up of the e.p.p. presumably is a period during which the transmitter continues to act; it appears that the duration of this action is greatly increased by a fall of temperature (see Discussion).

Furthermore, at the lower temperature the e.p.p. is reduced in size, on the average by about 40 per cent.

D. INTERACTION BETWEEN ENDPLATE POTENTIAL AND "ANTIDROMIC" SPIKE

To obtain further information on the nature of the e.p.p., its interaction with a propagated muscle impulse was studied. A volley of muscle impulses

Table 3. Effect of temperature on e.p.p. in the frog.[1]

	Time constant of decay of e.p.p. (fall to $1/e$)	Time of rise of e.p.p.	Time constant of decay of "supernormal period of 2nd e.p.p." (see Section E)	Conduction velocity of muscle	"neuro-muscular latency" (Section B)
Mean value at 20° C.	*11 msec.*	*2.3 msec.*	*39 msec.*	*1.6 m/sec.*	*1.35 msec.*
Extreme values at 20°C.	7–16.5 msec. (25)	1.65–3.5 msec. (25)	30–50 msec. (12)	1.2–2 m/sec. (20)	1.1–1.5 msec. (14)
Temperature coefficient Q_{10} (ratio for 10°C. difference in same muscle), mean value.	1.29	2.65 a) 2.7* b) 2.6**	3.8	2.09	2.1
Observed limits of Q_{10}	1.02–1.57 (5)	a) 2.1–3* (5) b) 2.15–3.5** (5)	2.6–5 (4)	1.9–2.6 (4)	1.95–2.2 (3)

[1] (Number of experiments shown in brackets.)
* Q_{10} for duration of rising phase.
** Q_{10} for reciprocal of maximum percentage rate of rise.

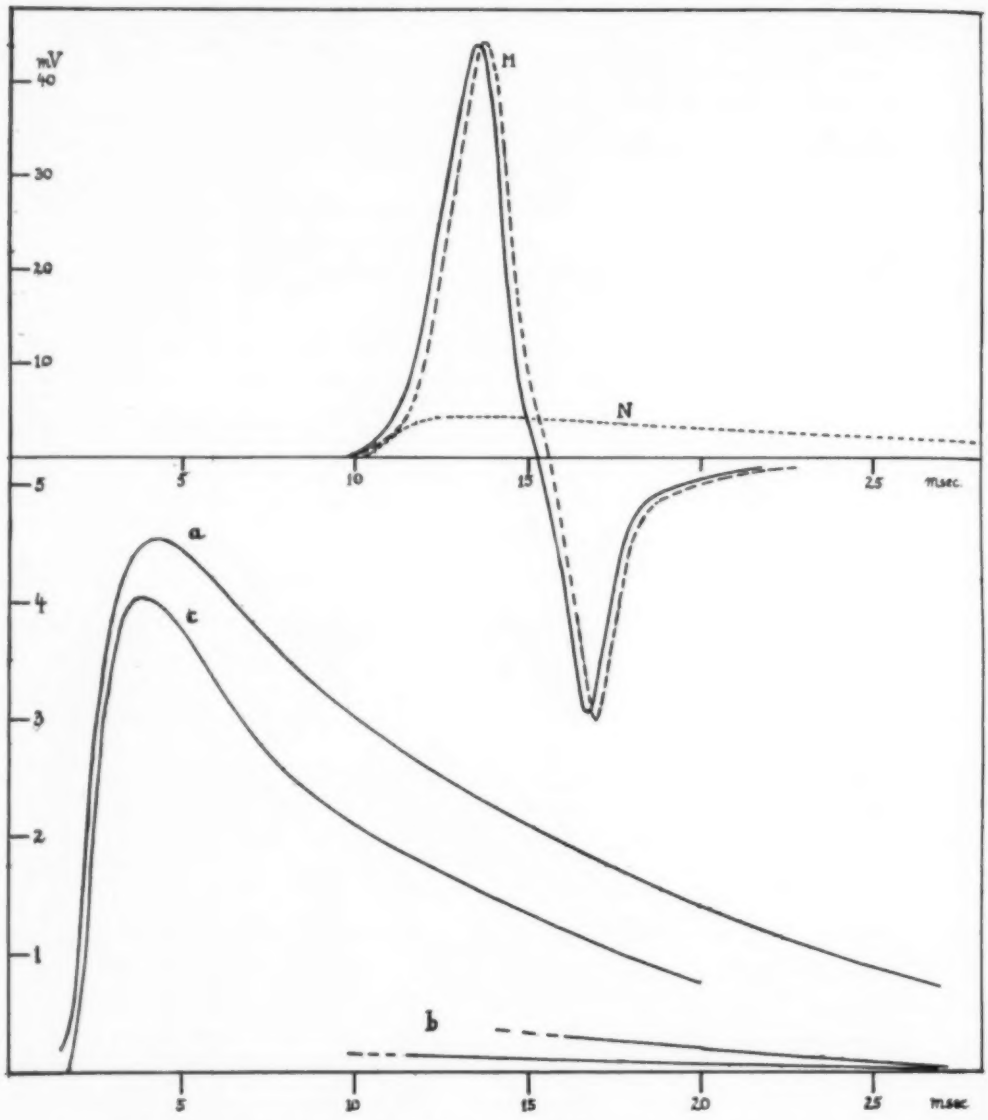

FIG. 9. Interaction between e.p.p. and muscle spike. Curarized frog's sartorius; 21°C. *Upper part:* Low amplification. Broken lines: *N*, e.p.p. due to nerve volley; *M*, diphasic spike due to direct stimulus. Full line: potential due to combined stimulation *NM*. Note, slight speeding of spike, no addition to spike peak. *Lower part:* High amplification. *a*, normal e.p.p. *b* and *c*, additions of e.p.p.'s during and after spike, obtained by curve subtraction ($MN - M$). *b*, remainder of e.p.p. set up simultaneously with spike (lower line), and 2 msec. before (upper line). *c*, e.p.p. set up 24 msec. after spike.

(henceforth called the "antidromic" volley) was set up in the frog's sartorius by stimulation at its nerve-free pelvic end and was superimposed at various time intervals on an e.p.p. (Fig. 9–12). Accurate investigation is only possible with large e.p.p.'s such as recorded from a sharply localized endplate focus in a state of critical curarization.

To summarize the main findings, (i) the antidromic impulse was slightly speeded up while travelling through the partially depolarized junctional region (Fig. 9, 10), (ii) the e.p.p. summed perfectly with the initial "foot" of the muscle spike, but added little (Fig. 11 B) or nothing (Fig. 10; 11 A) to its peak and descending phase.

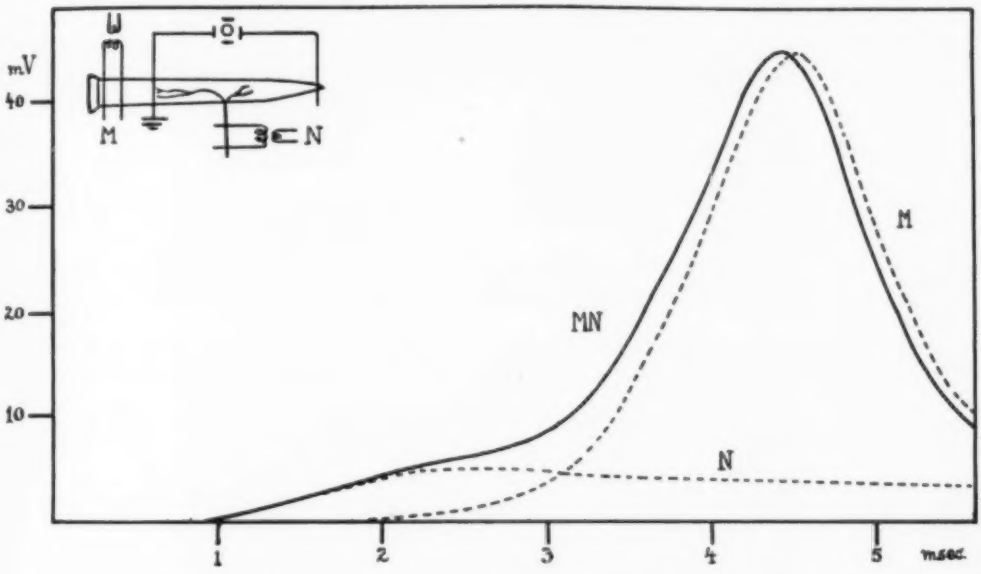

FIG. 10. E.p.p. and muscle spike. Frog's sartorius, at 22°C. Inset: preparation with stimulating (*N* nerve, *M* muscle) and recording leads. Broken lines: *N*, e.p.p. set up by nerve stimulus; *M*, antidromic spike set up by direct stimulus. Full line: potential change due to combined *MN* stimulus.

(i) *The effect of the e.p.p. on the propagation of the muscle impulse.* If the e.p.p. was set up simultaneously with, or a few msec. before, the arrival of the muscle impulse, it facilitated the propagation of the spike. The magnitude of this effect varied from experiment to experiment, depending no doubt upon variations in the local excitability of the muscle and in the degree of curarization. The maximum speeding was observed when the action potential was recorded at the tibial end, after it had travelled through all the "endplate zones" of the muscle, *i.e.* past two or three successive junctions in each individual fibre (cf. Katz and Kuffler, 1941). The greatest speeding amounted to 0.35 msec., equivalent to the conduction time for about 0.7 mm. of fibre length. It will be seen below that, when above a certain critical level, an e.p.p. gives rise to a propagated action potential; below this level, it exerts a subthreshold excitatory action on the muscle fibre, and this must be responsible for the speeding of the muscle impulse.

Though local depolarizations normally produce excitatory effects on nerve and muscle fibres, it has frequently been shown (*e.g.* Werigo, 1901; Blair and Erlanger, 1933, Fig. 11; Bugnard and Hill 1935; Katz 1937) that they exert inhibitory or even blocking actions, (i) when maintained at a high level for a long duration, (ii) when applied during a refractory

state. It is easy to understand, therefore, that the muscle impulse, while helped along by an ordinary e.p.p., is delayed or blocked by a cumulative and prolonged depolarization (Feng, 1937): the depression produced by an early second nerve volley (Eccles and O'Connor, 1939a; Eccles, Katz and Kuffler, 1941), and the well-known Wedenski-inhibition in normal and especially in eserinized muscle accompanied by a piling up of repetitive endplate potentials (Feng, 1939) are readily explained on this basis.

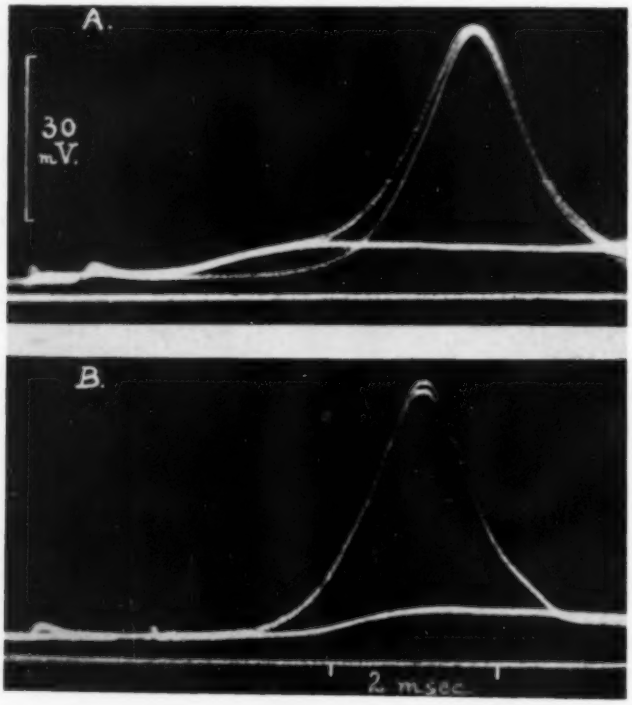

FIG. 11. Superimposed records of (i) antidromic spike, (ii) e.p.p., and (iii) combined potential change. Completely curarized frog's sartorius, at 22°C. *A:* Same as in Fig. 10. *B:* E.p.p. set up during spike. The e.p.p. gives a slight addition which rapidly disappears.

(ii) *The breakdown of the e.p.p. during the muscle spike.* As shown in Fig. 11 an e.p.p., if superimposed on an antidromic muscle spike, does not add its full height to that of the propagated potential wave. In fact, after an initial summation of the e.p.p. and the spike "foot" (Fig. 10), the e.p.p. seems to disappear completely, since neither the summit nor the descending phase of the action potential shows any addition or any alteration apart from a slight speeding. If allowance is made for this slight time shift of the later parts of the spike, the approximate shape of the superimposed e.p.p. can be obtained by a simple curve subtraction (Fig. 12). It appears that the e.p.p., after helping the antidromic impulse in its approach to the junctional region, is obliterated within 2 msec. during the ensuing spike process.

The recent work of Hodgkin (1937, 1938) and Cole and Curtis (1939) has shown (i) that the "foot" of the propagating action potential is identical with a subliminal depolarization of the axon membrane, and is caused by an

electrotonic spread of local action currents; and (ii) that at a certain critical potential level, "membrane excitation" occurs associated with a sudden breakdown of its transverse resistance. This change gradually dies away, as the membrane passes out of its refractory period. The present observa-

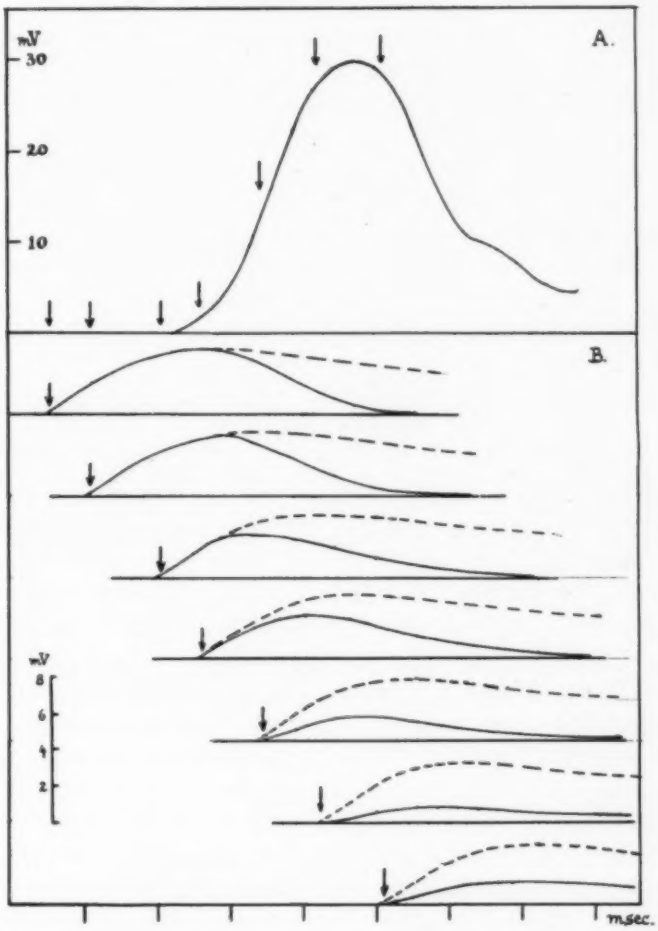

FIG. 12. Change of e.p.p. during passage of spike. Frog's sartorius at 22°C. completely curarized. *A*. Antidromic spike. *B*. Full lines: E.p.p.'s superimposed at various time intervals on the spike (see corresponding arrows in *A*). Broken lines: Control curves of e.p.p.'s alone.

tions are in good agreement with this concept. During the "foot" of the spike the two potential waves (e.p.p. and action potential) sum as two catelectrotonic potentials would do; as a consequence the critical threshold level is reached sooner and propagation speeded up. During the subsequent state of activity, the physical properties of the membrane undergo a sudden change which affects the size and time course of any superimposed electrotonic potential. As a result of the transverse resistance breakdown (Cole

and Curtis, 1939) the electric time constant of the membrane (*i.e.* the product *RC* of its transverse resistance and capacity) is greatly reduced, and any applied electric charge quickly leaks away. It follows that the decaying remainder of an e.p.p. rapidly collapses (Fig. 12). Further, a nerve volley arriving during this state of the membrane can only build up a much diminished e.p.p. (about 30 per cent, Fig. 12), just as in a non-medullated nerve fibre a stimulus, if superimposed on a propagating spike, produces a greatly diminished electrotonic potential (Hodgkin, 1938, p. 107).

If the nerve volley arrives at the myoneural region at progressive intervals *after* the antidromic impulse, the e.p.p. gradually recovers its normal size and duration. Even after 24 msec., however, the e.p.p. is depressed by 13 per cent below normal (Fig. 9). There is a possibility that this late depression might be only apparent, and really due to a slight movement of the muscle relative to the recording lead, but this has been checked by recording successively at a little distance to either side of the endplate focus.

It was observed in some cases that the atidromic spike left behind it a period of supernormal excitability during which neuro-muscular transmission was partially restored: in such preparations, for example, an e.p.p. set up 8 msec. after the antidromic spike was capable, in spite of its subnormal size, of eliciting propagated action potentials in some fibres.

The observed interactions between muscle spike and e.p.p. permit a number of inferences: they confirm the view that the e.p.p. is a subthreshold alteration of electric charge at the muscle membrane, and also provide evidence for an electric propagation of the muscle impulse in accordance with the Hermann-Lille hypothesis and Hodgkin's and Cole's recent observations on nerve.

Moreover, the fact that the descending part of the e.p.p. does not "survive" the passage of a muscle spike indicates that the depolarizing agent responsible for the *production* of the e.p.p. has ceased to act long before the latter has disappeared. If this were not so, the e.p.p. should be only temporarily depressed during the spike and afterwards built up again to its normal amplitude. This is indeed observed in the eserinized preparation (Eccles, Katz and Kuffler, 1941), where the depolarizing agent persists for a long period and so rebuilds the e.p.p. after the passage of the spike; in the same way, a catelectrotonic potential in a nerve axon recovers after the discharge of an action potential, provided the applied current is maintained (Hodgkin and Rushton, unpublished). In the curarized preparation, however, the remainder of the e.p.p. is permanently reduced to less than 20 per cent, or completely abolished, by an intervening spike (the small addition in Fig. 9 is probably due to uncorrected time shift of the *MN* curve). Thus it seems that most of the declining phase of the e.p.p. is a passive decay of a negative membrane charge after the depolarizing agent has ceased to act. The earlier suggestion, therefore, that the decline of the e.p.p. follows the time course of a passively decaying electrotonic potential (see p. 370) is confirmed.

This property of the e.p.p. differs markedly from that of a "negative

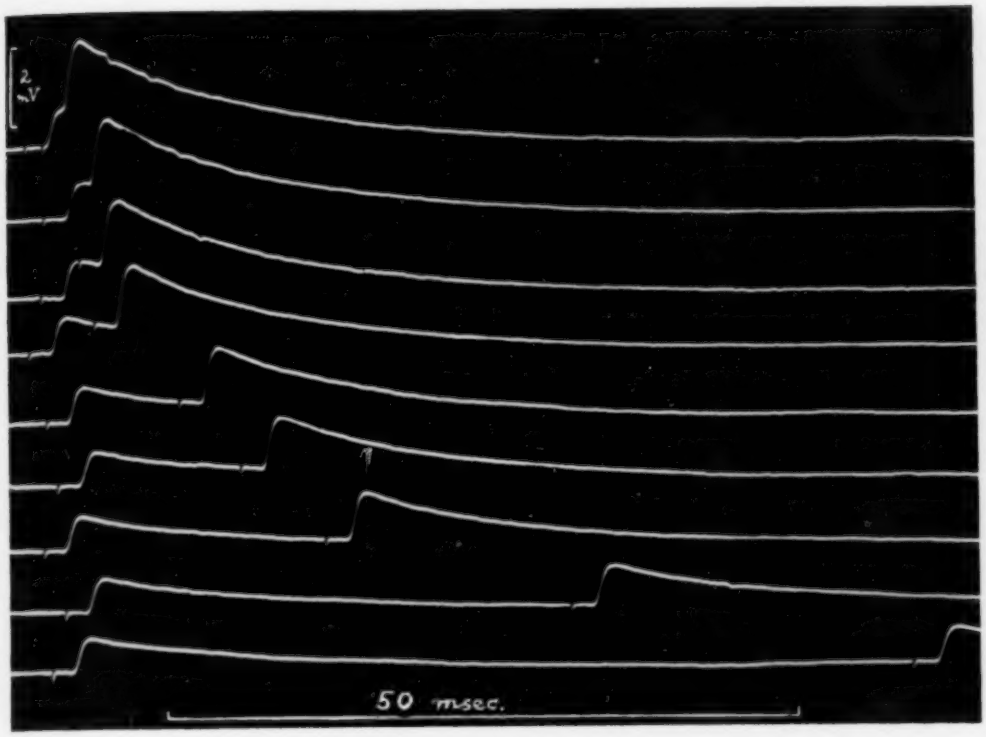

FIG. 13. Summation of e.p.p.'s with two successive nerve volleys. Frog's sartorius, at 25°C.

after-potential" which, as Gasser (in Gasser and Erlanger, 1937) has shown, sums with a subsequent spike and, to some extent, piles up during tetanic stimulation. This difference is of interest since there was previously some doubt (*e.g.* Katz, 1939) whether the e.p.p.—and its associated excitatory effect—is analogous to a "negative after-potential" extending to the myo-neural junction and associated with a super-normal phase of transmission, or—as supported by the present findings—to a "local potential" (Hill 1936) in the muscle fibres which declines slowly because of the long time constant of the muscle membrane.

E. THE MECHANISM OF NEURO-MUSCULAR FACILITATION

It has been shown in section *D* that the e.p.p. acts like a subliminal catelectrotonic potential of the muscle fibres and can add its excitatory effect to that of the subliminal current spreading in front of an advancing action potential wave. From this it may be inferred (i) that two e.p.p.'s set up by successive nerve volleys will sum as successive catelectrotonic potentials would do (see Schaefer and Haass, 1939), and (ii) that the e.p.p., if above a certain threshold level, must itself be capable of eliciting propagated action potentials. Furthermore, in a critical state of curare-block, neuro-muscular transmission should be restored by the summation (a) of successive e.p.p.'s

or (b) of an e.p.p. and a subliminal electric current pulse applied to the myoneural region, as was found by Schaefer and Haass (1939) and Katz (1938).

A. *Frog's muscle.* Summation of two e.p.p.'s in the frog's sartorius is illustrated in Fig. 13 and 14. These figures reveal an unexpected fact, namely that the e.p.p. produced by the second nerve volley is *greater* than the first

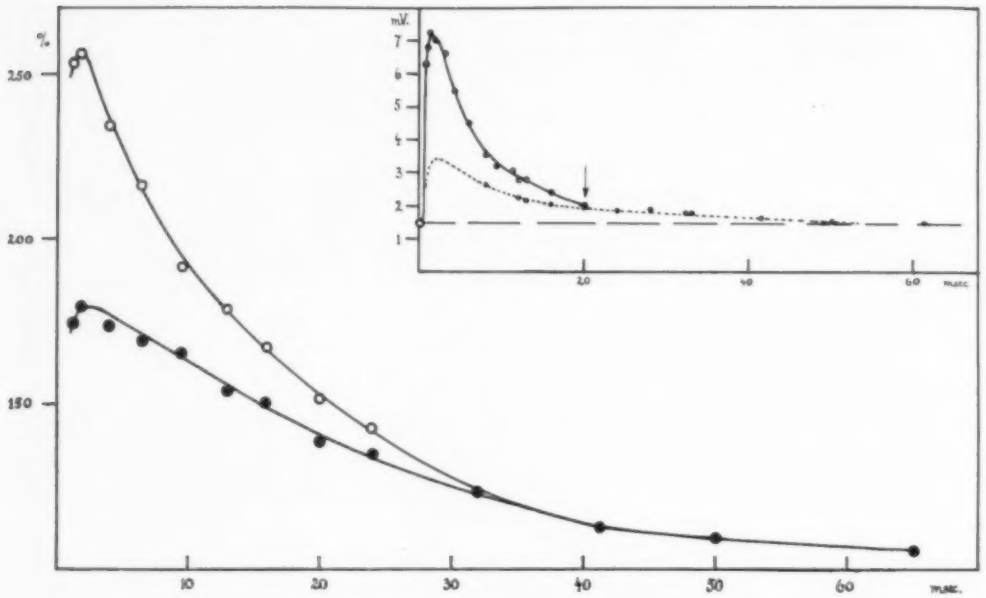

FIG. 14. Summation of e.p.p.'s with 2 nerve volleys (*cf.* records in Fig. 16B). Frog's sartorius at 25°C. completely curarized (6μmol. curarine). Abscissae: Time interval between nerve volleys. Ordinates: Size of e.p.p. in p.c. of single e.p.p. Hollow circles: total height. Full circles: height of added second e.p.p. INSET (*cf.* FIG. 16A): Facilitation in partially curarized muscle (3μmol curarine). Ordinates: Peak potentials. *Full line* (hollow circles): total potential height (spike +e.p.p.). Arrow indicates threshold level of e.p.p. (2 mV) at which spikes are initiated. *Broken line* (full circles): Height of summed e.p.p.'s. With intervals less than 8 msec., the e.p.p. was obscured by spikes. Broken curve shows height which summed e.p.p.'s would reach in absence of spikes (as with 6μmol curarine).

(Schaefer and Haass, 1939; Feng, 1940), though its time course is unchanged. The summed potential may reach 280 per cent, and the "supernormal" second e.p.p. 200 per cent of the height of the single e.p.p. (see Table 4). The

Table 4. Supernormal size of second e.p.p. in the frog's sartorius

12 experiments. at 23.5°C. (17.5–29°C.)	*Size, in per cent of single e.p.p.*	
	total height.	height of added 2nd e.p.p.
	264 (240–296)	188 (171–206)

Time factor k'' of decay of "supernormal period" (k'' = average time for fall to $1/e$): At 20°C., k'' = 40 msec.

Observed limits for k'': 8.5 msec., at 31.5°C.
190 msec., at 8.6°C.
Q_{10} = 3.8 (2.6–5, in 5 experiments).

"supernormal" effect dies out within about 0.1 sec. at 20 C. (Fig. 15). Its rate of decay has a remarkably high temperature coefficient, being slowed about 4 times by a 10 C. temperature reduction.

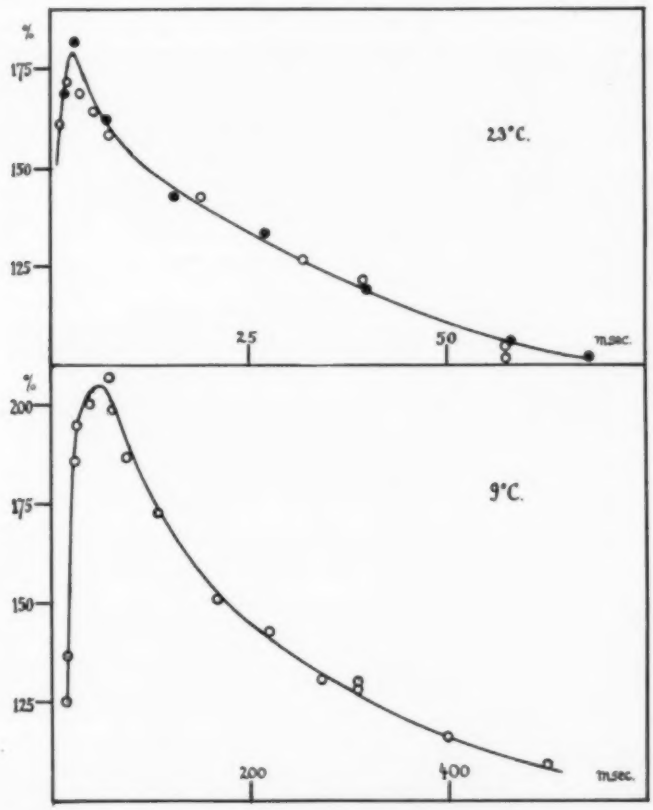

FIG. 15. "Supernormal period" of second e.p.p. Completely curarized frog's sartorius. Ordinates: Size of second e.p.p. in p.c. of the first. Abscissae: Time interval between two nerve volleys. *Upper part:* 23°C. 2 series of observations, before (hollow circles) and after (full circles) low temperature series. *Lower part:* 9°C.

With a smaller dose of curarine, summation of two e.p.p.'s may give rise to propagated spikes and mechanical twitches, i.e. to "neuro-muscular facilitation" (*cf.* Adrian and Lucas, 1912; Bremer, 1927). In the case of Fig. 16, a single nerve volley produces a subthreshold e.p.p. on which a second volley adds its e.p.p. after various intervals. Whenever the summed potential wave exceeds a level of 2 mV, propagated spike potentials appear. The observed size of the critical potential level at which muscle spikes are initiated depends, of course, upon the distribution of motor nerve endings: in preparations with a sharp endplate focus up to 4 mV (8 per cent of the spike) has been found (*cf.* section *B*). At the critical volley interval (Fig. 16) the spike is seen to take off at the peak of the e.p.p. With shorter intervals, the summed e.p.p. progressively exceeds the threshold level; the spike, there-

fore, becomes larger, and takes off during the rising phase of the second e.p.p. The critical threshold is illustrated even better on the strip preparation of the cat (Fig. 18), where the spike is simple and synchronous (Eccles and O'Connor, 1939).

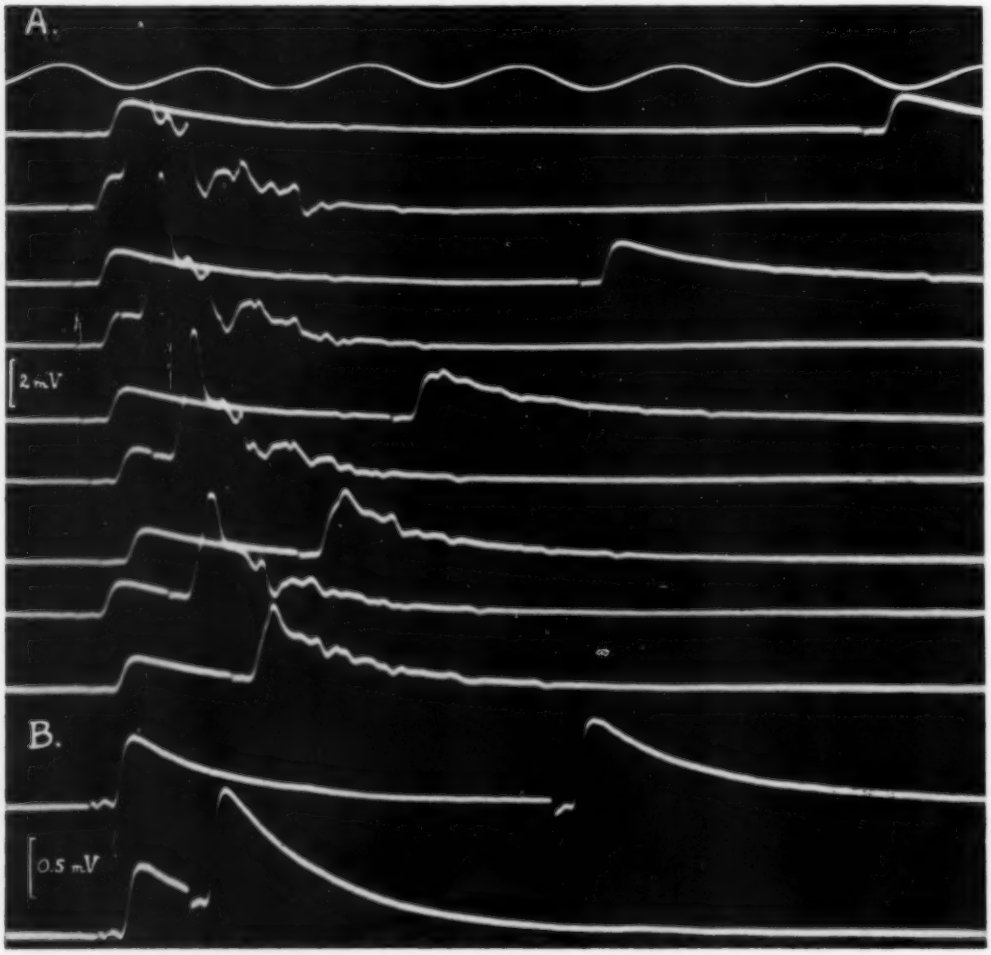

FIG. 16. Neuro-muscular facilitation. Frog's sartorius at 25°C. *A*. 3μmol curarine. Appearance of spikes with two nerve volleys, if e.p.p. exceeds about 2 mV. (*cf.* INSET, FIG. 14). *B*. 6 μmol curarine. Summed e.p.p.'s do not reach threshold. Time signal: 1 d.v. = 10 msec.

The irregular polyphasic shape of the spikes in the partially curarized sartorius (Fig. 16) is due to their scattered points of origin along about 2.5 cm. of muscle (Katz and Kuffler, 1941). In the normal preparation the late spikes are not recorded because they collide with impulses arising, in the same muscle fibres, from the endplate nearest to the recording lead (Katz and Kuffler, 1941). Furthermore, in a critical stage of curarization, single fibre impulses sometimes arise quite late—on the falling phase of the e.p.p. Such long latencies might be due to a local response (Hodgkin, 1938) which at threshold level may linger on for a few milliseconds before giving rise to a propagated spike.

The magnitude and time course of neuro-muscular facilitation in the frog depend upon two factors: (i) upon the size and time course of decay of the first e.p.p., on the declining remainder of which the second e.p.p. is added, (ii) upon the supernormal size of the second e.p.p. This supernormal effect outlasts the single e.p.p. and, therefore, a remainder of neuromuscular facilitation can persist beyond the period during which there is actual summation of the two e.p.p.'s.

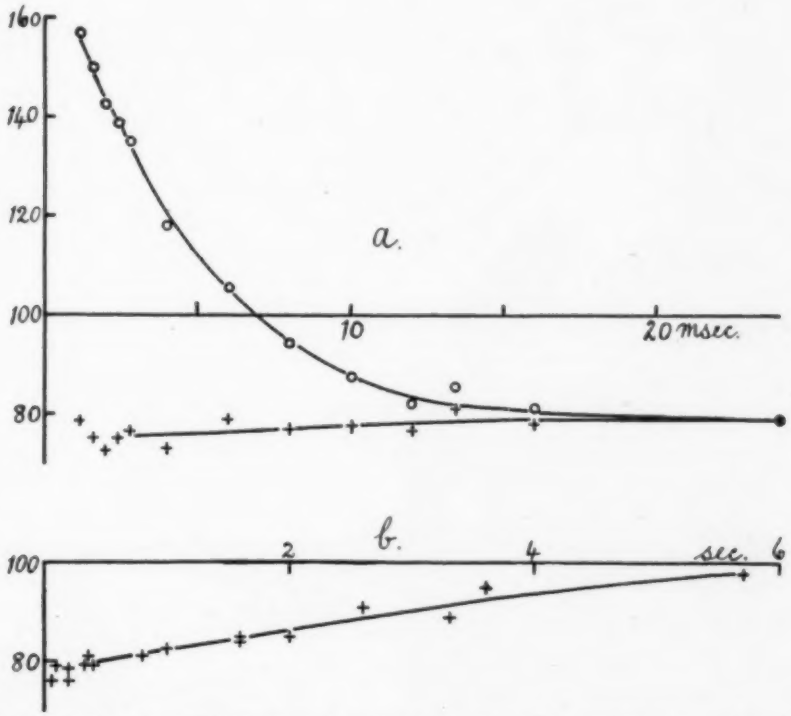

FIG. 17. *Cat's soleus.* Plotting as in Fig. 14 of e.p.p.'s set up by a second nerve volley (a) Circles: total size. Crosses: size of added second e.p.p. (b) shows recovery of added second e.p.p. at longer volley intervals.

At about 30°C., the e.p.p. set up by the first nerve volley and the supernormal phase of the second e.p.p. subside at about the same speed, while at 10°C., owing to the different temperature coefficients of the two processes, the supernormal effect decays about 9 times more slowly than the first e.p.p. Hence, as the temperature is lowered, the duration of neuro-muscular facilitation progressively exceeds that of the single e.p.p.

Thus, the earlier suggestion (Katz, 1939), *viz*, that the gradual subsidence of neuro-muscular facilitation is due to the electrotonic decay of a local depolarization of the muscle fibres is only partly confirmed; the "supernormal" size of the second e.p.p. is an additional factor at least in frog's muscle. The nature of this supernormal effect cannot at present be explained, but it is interesting to note that the effect is greatly reduced, or even abolished, by eserine, as will be reported later.

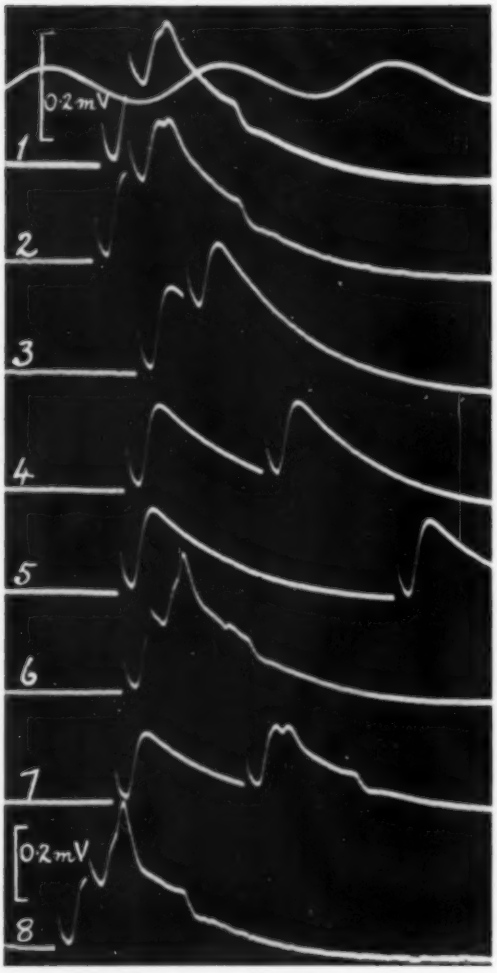

Fig. 18. *Cat's soleus.* E.p.p.'s set up by 2 nerve volleys at indicated intervals apart. *1* and *2:* summed e.p.p. reaches 0.26 mV and a small muscle spike (possibly in a single fibre) arises at the summit. *3:* A slightly lower e.p.p. (0.25 mV) fails to set up a spike. *6*, *7* and *8:* less deep curarization (single e.p.p. 0.205 mV; 0.165 mV for earlier series) and correspondingly the small spike is set up even at 8 msec. interval (summed potential 0.24 mV). The larger e.p.p. at shorter intervals (at least 0.32 mV in *6* and *8*) sets up impulses in additional fibres as shown by the larger spike. Thus 0.24 to 0.25 mV is the critical e.p.p. for setting up spike. Time: 1 d.v. = 10 msec. Potential scales shown for 1st and 2nd series. Apparent positivity at end of records due to amplifier distortions.

In some experiments there was evidence of a protracted subnormal period, with intervals greater than 0.15 sec., though the depression did not exceed 5 per cent.

B. *Cat's muscle.* In soleus (also peronaeus tertius and tibialis anticus) the second e.p.p. is always *diminished* by about 80 per cent (70–95 per cent). Figure 17 shows that this depression persists for a few seconds—full recovery requiring 2.5 to 10 sec. As a result of this depression, neuro-muscular facilitation in the cat is much less conspicuous than in the frog. The summed e.p.p.'s reach a maximum height of only about 170 p.c. of the single e.p.p., and the period of facilitation is terminated at an interval of about 8 msec. (6–20 msec., *cf.* Fig. 17 and 18). Beyond this interval, the summed potential is smaller than the first e.p.p. In partially curarized muscle, therefore, the spike response to a second nerve volley is diminished at such intervals (*cf.* Brown, 1938).

Discussion

The nature of the e.p.p. The present experiments have shown that a nerve impulse arriving at a curarized myoneural junction sets up a local depolarization in the muscle fibre (the "endplate potential") which acts in all respects like the local potential produced by a subthreshold electric stimulus. The evidence relating to this may be summarized as follows.

(1) The e.p.p. spreads along the muscle with a decrement of about 50 p.c. per mm., and with progressive slowing of its time course (Section A).

(2) The time factor involved in the decay of the e.p.p. (Section B) is of the same order as that of the catelectrotonic potential (Schaefer and Haass, 1939) or the α-excitability of muscle (Rushton, 1930). This time factor has a low temperature coefficient ($Q_{10} = 1.29$) as expected for a passive electrotonic decay (Section C).

(3) The e.p.p. sums with the initial "foot" (*i.e.* the extrinsic passive phase) of an approaching muscle impulse just as a catelectrotonic potential would do, and the impulse propagation is speeded up (Section D).

(4) During the "active phase" of the muscle spike (*cf.* Hodgkin 1937, 1938), the e.p.p. resembles a catelectrotonic potential in that it is built up to a much smaller potential and its decay is much more rapid (Section D).

(5) If the e.p.p. exceeds a certain critical level, a muscle impulse is initiated (Section E). Facilitation of neuro-muscular transmission occurs when summation of successive e.p.p.s gives a sufficiently high level (Section E).

The transmitter. The present work does not indicate how the endplate potential is produced. It might be due to the depolarizing influence of local action currents, or to a transient effect on the muscle membrane by a chemical transmitter such as acetylcholine. Our present knowledge concerning the mode of action of curare appears to favour the acetylcholine theory. Curare greatly diminishes the stimulating action of nicotine (Langley, 1909) and of acetylcholine on skeletal muscle (Brown, Dale and Feldberg, 1936), and this parallels its powerful action (Section A) in diminishing the endplate potential, *i.e.* the depolarizing effect of the transmitter. Furthermore, Lapicque's evidence that curare alters the electric excitability of the muscle can now be considered as disproved (see Rushton, 1930; Schaefer, Schölmerich and Haass, 1938). However, it may well be that curare has various other actions at the myoneural junction (*cf.* its action on nerve: Fromherz, 1933; Fessard, 1936). Further discussion of the transmitter problem will be deferred to a later paper dealing with the action of eserine.

Time course of transmitter action. Whatever may be the nature of the agent producing the endplate potential, one can determine the probable time course of its action. It has been shown that most of the declining phase of the e.p.p. is a period of passive electrotonic decay. Since it follows an approximately exponential time course, one can analyze the e.p.p. on the basis of Hill's "local potential" theory (1936) and so obtain the probable time course of its underlying cause.

Making assumptions analogous to Hill's (1936), the endplate potential P, at any moment t, builds up at a rate (dP/dt) proportional to the intensity of the transmitter action cA at that moment (c being a proportionality constant), and simultaneously tends to decay exponentially with time constant k. P, k and dP/dt being known, cA can be calculated from

$$dP/dt = cA - P/k \qquad (1)$$

In Fig. 19 the time courses of the e.p.p. and the depolarizing agent, as obtained by analysis according to equation *1*, are illustrated. The duration of the depolarizing agent hardly exceeds the rising phase of the e.p.p., *i.e.*

about 2–3 msec. at 20°C. It will be shown in a later paper that eserine lengthens this period by more than 100 per cent.

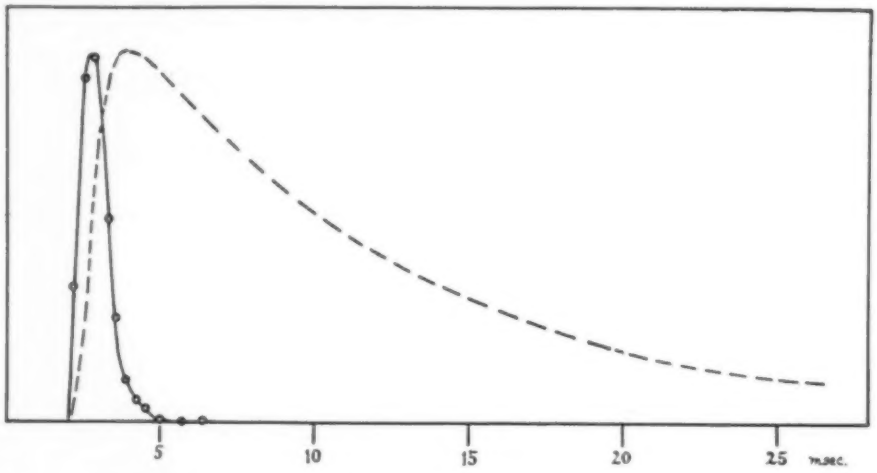

FIG. 19. End plate potential and transmitter action. Broken line: e.p.p. of frog's sartorius, at 17.5°C. Time constant k of decay (time for fall to $1/e$) =9.8 msec. Full line: Probable time course of "transmitter action," obtained by analysis according to equation (1). Ordinates in arbitrary units. Abscissae: Time after nerve stimulus.

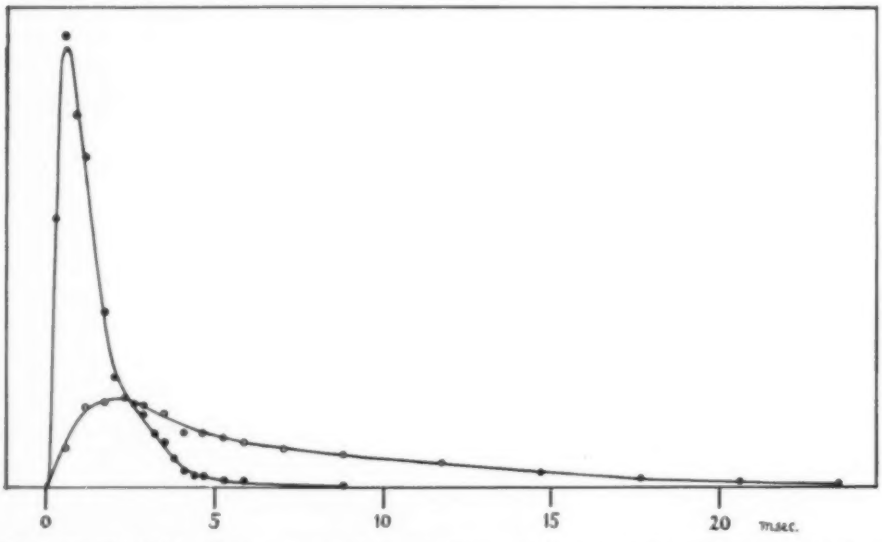

FIG. 20. Probable time course of transmitter action of 19°C. and 9°C., obtained by analysis of the e.p.p.'s of Fig. 8, taking into account the difference in amplitudes. Full circles: 19°C. Hollow circles: 9°C.

In Fig. 20 the e.p.p. has been analyzed at two different temperatures (*cf.* Fig. 8 above). The time course of the transmitter action, especially the rate of its decay, is greatly slowed at the lower temperature. Further, the

intensity of transmitter action is greatly diminished at the low temperature. The large temperature coefficient for the rising phase of the e.p.p., and so for the time course of transmitter action, might be taken as a further indication of its chemical nature. It should be remembered, however, that the duration and conduction velocity of the nerve or muscle spike have also a relatively high temperature coefficient (Q_{10} about 2.0, but occasionally reaching 2.6, *cf.* Table 3).

The analysis by equation (1) is open to certain objections, as it does not consider changes in the resting properties of the muscle membrane associated with the production of the e.p.p. This could be neglected only if the e.p.p. were set up by extrinsic action currents which would partially discharge the membrane without altering its permeability. If acetylcholine, however, is the responsible agent, its depolarizing action would presumably be accompanied by a diminution in membrane impedance, and this would complicate the analysis. But even then the membrane must have returned to its resting state after a few milliseconds, as is indicated by the exponential decay of the e.p.p. and by the fact that a second e.p.p. can be added, at any moment of the descending phase, without alteration in time course (Section E).

Length of muscle fibre affected by transmitter action. Owing to electrotonic potential spread, a considerable portion of muscle is affected by the local changes produced by the transmitter. Though the direct action of the transmitter would be restricted presumably to an area not larger than the end-plate, the depolarization spreads over a length of about 2 mm. Moreover, in the frog's sartorius, most fibres are supplied by 2 or even 3 discrete motor nerve endings (Katz and Kuffler, 1941), so that in a muscle of 35 mm. length about 15 p.c. of the fibre length is affected by local events at the junctional regions. It is not surprising, therefore, that under certain conditions, when large and prolonged e.p.p.'s are set up (high-frequency nerve stimulation; eserine; see Feng, 1937; Eccles, Katz and Kuffler, 1940), the junctional change may be accompanied by a measurable increase in mechanical tension and heat production of the muscle (Feng, 1937; Cowan, 1940).

Summary

In curarized frog's and cat's muscle a non-propagated negative potential change—the "end-plate potential"—is set up by a motor nerve volley at the region of the myoneural junctions.

By various tests, the end-plate potential (e.p.p.) is shown to be a local depolarization of the muscle fibres which acts like a local catelectrotonic potential produced by a subthreshold electric stimulus.

The e.p.p. spreads a short distance along the muscle fibres, with a decrement of about 50–75 per cent per mm., and a progressive slowing of its time course.

If recorded from the centre of an end-plate zone, in a state of just complete curarization, the e.p.p. reaches 5 to 8 per cent of the normal propagated muscle spike.

It waxes and wanes along an approximately double-exponential curve, the duration of the rising phase being 2.3 msec. in the frog's sartorius at

20°C., and 0.8 msec. in the cat's soleus, while the time constant of its descending phase is 11 msec. and 5 msec. respectively.

The temperature coefficient for the time factor of decay is low ($Q_{10} = 1.3$), that for the duration of the rising phase is high ($Q_{10} = 2.65$). The significance of this difference is discussed.

There is interaction between an e.p.p. and a propagated muscle-spike set up by direct stimulation. The e.p.p. sums with the "foot" of the muscle spike and slightly speeds up its propagation. During the "active phase" of the spike the e.p.p. is reduced or abolished.

In the frog a second nerve volley, at a brief interval after the first, sets up an e.p.p. which is up to 80–100 per cent larger than the first. This "supernormal" effect gradually disappears within about 0.1 sec. at 20°C. In the cat, the second e.p.p. is about 20 per cent smaller than the first and recovers its normal size only after several seconds.

If the e.p.p. exceeds a certain critical level, muscle spikes are initiated. Neuro-muscular facilitation occurs when the required level is reached by summation of successive e.p.p.'s.

We wish to thank the National Health and Medical Research Council of Australia for equipping and maintaining the workshop in which most of the apparatus was made.

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ISOLATION OF INTRINSIC AND MOTOR MECHANISM OF THE MONKEY'S SPINAL CORD*

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THE INTRINSIC MECHANISM of the spinal cord is compounded of nerve cells and nerve fibers: all the nerve fibers which both originate and terminate within the cord, and all the nerve cells giving rise to those fibers. The fibers in question for the most part compose the ground bundles or proprius system of the cord, running courses believed not to exceed seven segments up or down from their level of origin. But any of the long ascending fibers which send collaterals to the spinal grey matter must also be reckoned within the mechanism to the point, at least, where the last such collateral is given off. Correspondingly, the cells include small cells such as those in the substantia gelatinosa and the various sized association cells scattered throughout the grey which probably give rise only to intrinsic fibers, together with the larger cells which give rise to the long ascending systems but whose spinal collaterals enter into the system. Additionally, if any of the emerging ventral root fibers send recurrent (Golgi) collaterals back to the grey matter, then their cells of origin and fibers out to and including the collaterals form a part of the intrinsic mechanism. Excluded are the long descending systems, the entering fibers of the posterior roots, and the cells and fibers for the anterior roots when no collaterals are present.

To isolate the entirety of the intrinsic mechanism of the cord would require transecting the neural axis at the end of the medulla and severing all posterior and anterior spinal roots. This is scarcely practicable. To isolate a portion of the mechanism requires, however, only a double transection of the cord (one section high, one section low), and severance of the roots between. If only the sensory roots be cut, the motor roots being left intact, a preparation might be obtained in which many problems of function and structure in the intrinsic cord mechanism could be investigated provided only that the isolated region of spinal cord survived. That it can survive both the considerable trauma of the operation and being so isolated for a period of months has already been demonstrated for puppies (Tower, 1937), and some of the problems have been examined. The present report deals with a repetition of the experiment on 3 nearly grown monkeys (*Macaca mulatta*), killed, however, after 10 days or 2 weeks for chromatolysis studies on the isolated cord segments.

The surgical procedure consisted in exposing the lumbo-sacral spinal cord by removing the vertebral spinous processes and slitting the dura. The cord was then transected between the last thoracic and first lumbar segments and again below the sacral region, and

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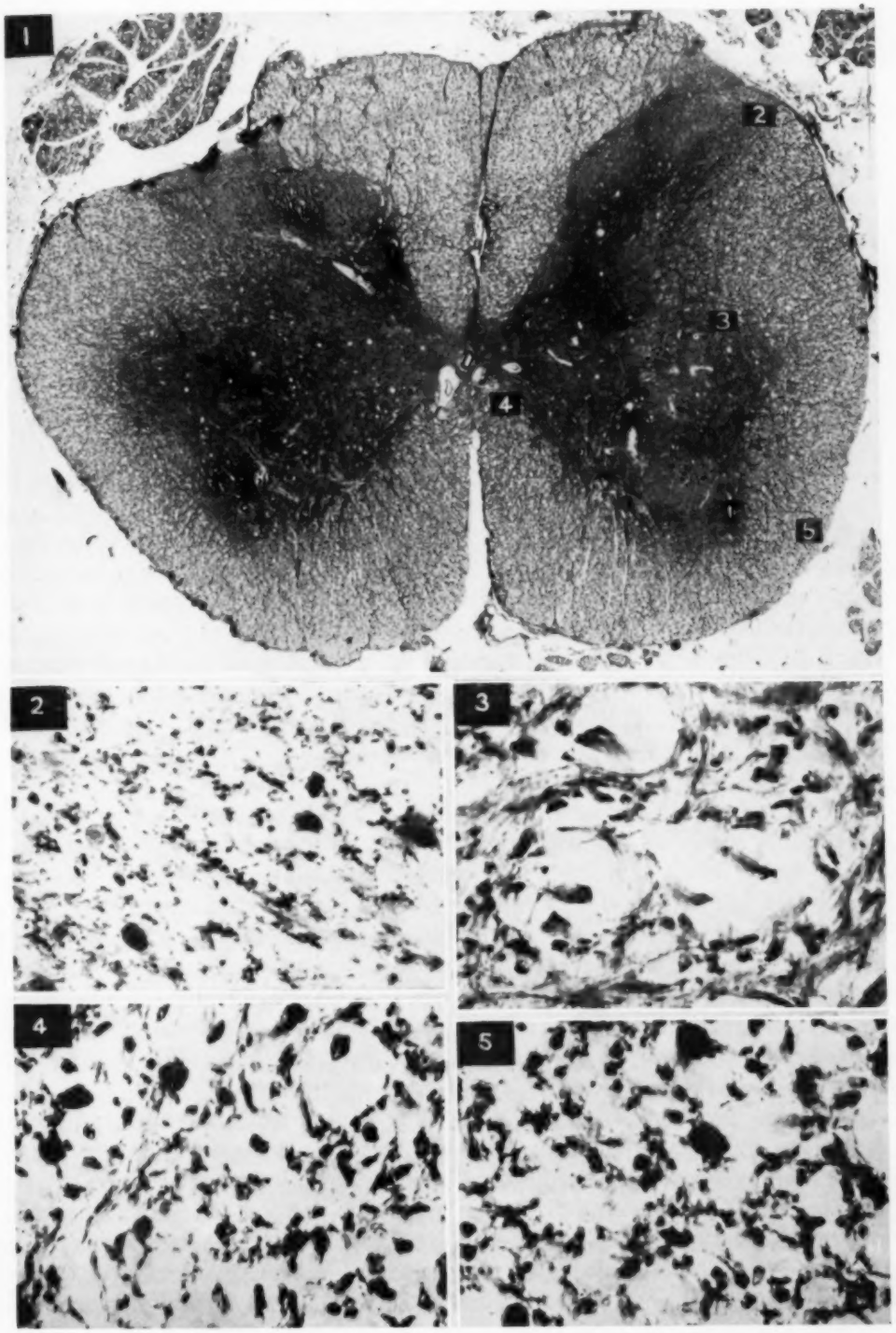


FIG. 1. Microphotograph of a section from the L_6 level of the completely isolated cord segment, stained with silver.

FIG. 2–5. Sample areas from Fig. 1 as indicated; oil immersion; ×870. (2) from the lateral extension of Lissauer's zone; (3) from the lateral reticular formation; (4) from the deep end of the anterior column; (5) from the surface zone of the anterolateral column.

all intervening dorsal roots were cut intradurally on both sides, carefully sparing the anterior roots. The anterior spinal artery was preserved intact. Examined at autopsy, the isolated lumbo-sacral region of cord was found to be healthy and without blemish other than the intended sections in 2 monkeys, but liquefied in the third. In both surviving cords the upper transection fell between the T_{12} and L_1 segments and was complete, but the lower section was incomplete in one of the pair. This animal was killed on the 10th, and the other two on the 14th day of survival. In all 3 animals on the day following the cord operation the right sciatic nerve was exposed and cut near the greater trochanter without benefit of anaesthesia and without evidence of sensation on the animal's part. All the monkeys made uneventful postoperative recoveries, eating well and moving as much as their condition permitted. In spite of nursing care ulcerations developed in a number of sites, the largest over the right greater trochanter. Bladder and rectum had to be emptied by pressure. Terminally, under ether anaesthesia, the animals were killed and fixed by intravascular perfusion of 100 cc. of saline followed by 1 per cent acetic acid in 10 per cent formalin.

Examined in the interval of survival, all 3 monkeys were completely anaesthetic over the hind quarters, with a sensory level at the umbilicus or just below. In the animal which had an incomplete lower cord section, tail, perineal and genital reflexes were obtainable, but leg reflexes were absent. The other two animals yielded no skeletal reflexes whatever below the level of anaesthesia. The phrase "complete flaccid paralysis" describes the condition of the skeletal musculature innervated from the isolated segment, for no activity of any sort, neither tonic nor phasic, was observed in the musculature at any time after the operation, and no distinction could be made between the side with additional sciatic section and that on which the sciatic nerve remained intact. Moreover, prior to the final exposure of nerves and muscles there was no indication that the isolated segments of cord had survived the operation variously. Fasciculation was not observed in the affected skeletal musculature.

The two surviving isolated regions of cord were made the object of histological study in 10μ sections prepared both by Einarson's gallocyanin technique for Nissl substance and by Bodian's silver method for axis cylinders. From both cords the L_5 to L_7 segments were cut serially, transversely. From the completely isolated cord additional specimens from the lower part of T_{12} and the upper part of L_1, that is just above and below the upper section, and a specimen from S_2 were cut transversely, and the whole L_4 segment horizontally. Similar transverse sections from normal monkey cords were available for comparison, together with an L_4 specimen obtained eight days after a lateral hemisection of the cord at the C_1 level.

Outstanding on first examination of the sections, L_1 excepted, are their good form both exteriorly and interiorly, and their approximately normal size. Later, presumably, shrinkage would have followed on the destruction of fibers, but the debris of that destruction is not yet entirely cleaned up, nor is sclerosis under way. Neuroglia are, however, unusually numerous and conspicuous in both white and grey matter. Figure 1 reproduces a cross section from the upper end of L_6 stained with silver. Although the trifling incompleteness of the caudal transection in one animal of the pair makes no noticeable difference at thè levels examined, nevertheless both description

and illustrations are taken from the completely isolated specimen. The L_1 specimen, in contrast with the lower levels examined, is flattened and shrunken, with few cells surviving though many fine fibers, and with much evidence of widespread destruction.

Examining the silver preparations for nerve fibers:—The posterior roots are cut and degenerated proximally to the cut, but the anterior roots are intact. Occasional degenerated or degenerating fibers may be found in these roots but for the most part the fibers appear healthy. Within the cord the number of fibers surviving is so great that loss of fibers is at first glance scarcely appreciated until large myelinated fibers are sought. Healthy appearing fibers are distributed everywhere throughout the white matter, though far from evenly. Figure 6 represents diagrammatically the relative density of this distribution. Fibers are sparsest in the dorsolateral region of the posterior column where the medial division of the posterior root debouches into the cord. Indeed, this region is almost cleared of fibers. Next most depleted, but still possessing many small fibers, is the surface zone along the ventromedial curvature of the anterior column. The middle part of Lissauer's zone, the dorsal portions of the posterior columns, and the area occupied by the long descending motor systems of the lateral columns have also lost heavily in fibers. Adjacent to the grey matter everywhere except along part of the medial border of the posterior horn are fairly dense concentrations of fibers, occupying the dorsomedial third of the anterior column throughout its width, and extending to the surface of the cord in the ventrolateral region. The densest concentrations of fibers are found in three sites; lateral and medial to the tip of the posterior horn, although the small collection medial to the tip is not continuously present, and at the deep end of the posterior column. The large collection lateral to the tip of the posterior horn is the densest fiber field remaining in the cord.

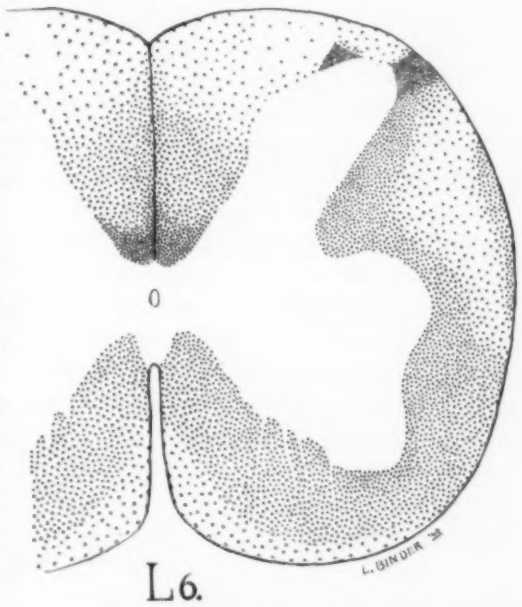


FIG. 6. Diagram representing the relative density of distribution of fibers surviving in the white matter of the completely isolated segment of spinal cord.

The vast majority of the surviving fibers are small and seemingly unmyelinated, and for the most part they are clustered in the framework left after degeneration of the large myelinated fibers. The two dense areas at either side of the posterior horn are composed of the most minute fibers, most

of them well under 1μ, and here the fibers are so closely and evenly packed that it seems unlikely these areas have suffered much, if any, fiber loss. Throughout the posterior column the fibers are very little larger, averaging around 1μ, but with a scattering of larger fibers, and the same is true of the dorsal parts of the lateral column. In contrast, beginning with the reticular formation in the angle between the dorsal and ventral horns, and bordering the grey matter around to the anterior commissure, the surviving fibers are of all sizes, ranging from a fineness almost too minute to be measured to fibers nearly as large as any to be found in the intact cord. Although the disappearance of many large fibers and consequent loose disposition of the survivors makes measurement difficult, diameters range from about 0.5 to 15μ for the poorly defined outside sheaths, and from 0.5 to 6μ for axis cylinders. The larger fibers are, of course, unmistakably myelinated, but myelin space may be recognized, though emptied in these silver preparations, on axis cylinders of 2μ diameter and perhaps less. Accompanying the cross section of the cord of Figure 1 are sample areas (Fig. 2–5) showing the characteristic fiber composition in four regions of the white matter.

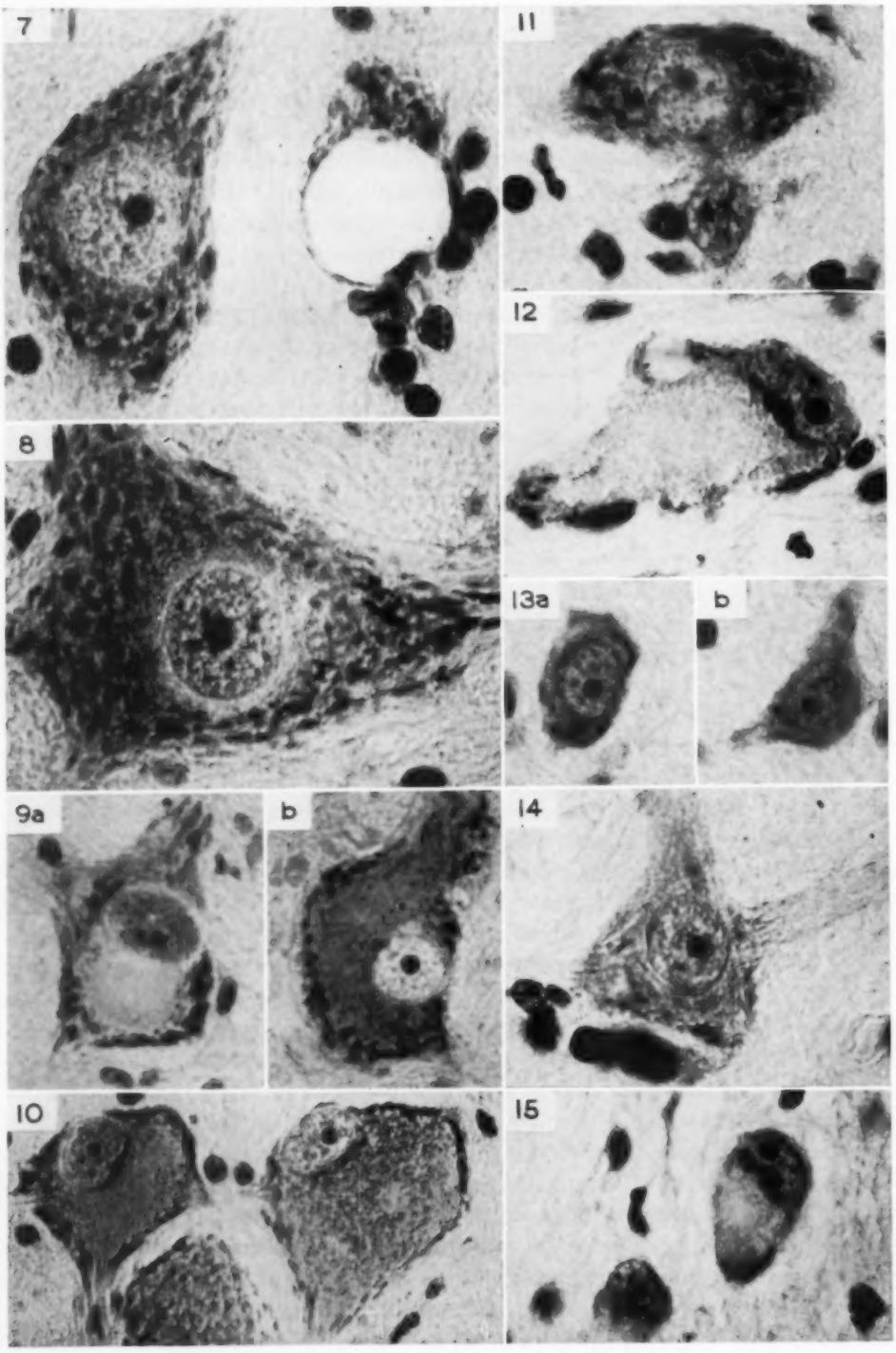
The presence of large fibers, fibers in excess of 10 or 12μ diameter, in the isolated length of spinal cord is noteworthy, especially so in view of Lloyd's (1941) demonstration that propriospinal fibers, as he calls them, with alpha conduction rates participate in aboral conduction within the spinal white matter. All levels examined except the much damaged L_1 level contain such fibers, although they are few at the S_2 level. Throughout the lumbar levels they constitute a numerous and impressive total aggregate. Two groups of these large fibers are outstanding; one in the reticular formation adjacent to the dorsolateral border of the anterior horn (Fig. 3) and extending around its dorsolateral tip; the other in the deep third of the anterior column (Fig. 4), beneath the anterior commissure. These latter fibers are traceable in some instances from the commissure, most clearly in the horizontal sections. Similar fibers are almost as numerous, cut in cross section, adjacent to the grey matter between the bundles of emerging ventral root fibers, but fewer along the lateral border of the ventral horn below the reticulated region. And in the horizontal sections the straight courses of individuals among these large fibers can often be followed for several millimeters, long enough to ensure that they are not emerging ventral root fibers. The course pursued by these large fibers is commonly lengthwise of the cord, but the direction, whether ascending or descending, is not evident. Different fibers may well course is opposite directions, and even one fiber in both directions if it is constituted as ascending and descending branches of a parent stem. Almost all these large fibers are found close to the grey matter, suggesting that whether or not any of them run through to the brain as a long ascending system, they all function importantly in the intrinsic cord mechanism. Throughout the superficial portions of the ventral and lateral white matter only an occasional large myelinated fiber is to be found, and the few present in the posterior columns lie deep, adjacent to the posterior commissure.

Similar large myelinated fibers, the largest, in fact, a shade larger, were described in the puppy cords isolated for months. There the fibers were distributed essentially as in the monkey except for more even scattering through the anterior and lateral columns adjacent to the grey matter, with an outstanding group only in the lateral reticular formation.

The fields of fibers beneath the surface of the lateral and ventrolateral white matter, which are fairly dense in the lumbar levels but sparse in the sacral, present a problem. They are composed almost exclusively of small fibers,—both unmyelinated fibers in clusters, and small myelinated fibers up to 5 or 7μ over-all diameter (Fig. 5). Judging by the density of these areas in the isolated segments as compared with normal material, many of these fibers especially the myelinated individuals, must be components of the long ascending sensory systems which in the intact cord concentrate in these sites. Such fibers could very well survive up to the superior transection, for as will be shown later their cells of origin are largely intact. To what extent such fibers are to be considered components of the intrinsic system as originally defined, would be determined by whether or not they give off collaterals to the grey matter in their upward transit. Although this seems quite possible, the confusion of fibers is too great to permit of settling this point in reduced silver preparations.

Turning from the white to the grey matter but continuing the examination of fibers:—The neuropil appears, by comparison with normal material, definitely reduced in density. Outstanding even in Fig. 1 is the elimination of the heavy strands of root fibers or their collaterals which normally sweep into the dorsal horn from the dorsal root and column, but the intervening feltwork of fibers of the posterior horn is also unmistakably impoverished. Thinning is less noticeable in the anterior horn. Nevertheless the impressive aspect of the neuropil in both these sites is the number of fibers, both axonal and dendritic, surviving. And these fibers appear quite healthy and of a normal range of size except for certain pathological changes in the large dendrites to be described shortly.

The cells of the cord have for the most part also survived; and in larger proportion, on close scrutiny, than the fibers. But whereas the surviving fibers appear in all respects normal, the surviving cells are in many instances clearly pathological. Two types of disorder are in evidence. The more conspicuous is a condition of vacuolation barely visible in Fig. 1; the more subtle, chromatolysis in varying degree. These are distributed seemingly quite independently of one another throughout the grey matter. The vacuolated condition attacks only large cells, and in flagrant form, only anterior horn cells of the motor type, but attacks erratically so that two individuals lying side by side as in Fig. 7 may be: the one quite unaffected, the other almost overwhelmed by the process. No particular group of motor type cells is selected. The process consists in the formation of one or more large vacuoles, some as big or bigger than the nucleus, which may be located anywhere within the cell body or its larger dendrites, even bulging from the surface.



FIGS. 7–15

If the vacuoles are fairly small or few, the internal organization of the cell is not otherwise disturbed. Nissl bodies and neurofibrillae may be clear, and the nucleus normal in form and location. Well-formed Nissl bodies can be seen concentrated outside the vacuole in Fig. 7. Here and there, however, are frankly disintegrating cells, or the residue of completed cell destruction in accumulations of small round cells, probably phagocytes. Vacuolation was also the type of cell pathology present in the puppy cords after months of isolation, but its occurrence after other lesions, for example on both sides at the L_4 level after hemisection at C_1, and after root or even peripheral nerve sections, makes its interpretation obscure.

Chromatolysis is much more widespread throughout the isolated segments, attacking large and medium sized cells of both anterior and posterior horns in varying severity. Three types of distribution are present in the anterior horns. On the right side of the sections the proper cell groups in the L_{5-7} segments exhibit the full-blown central chromatolysis with displacement of the nucleus to the periphery illustrated in Fig. 10, and to be expected 13 days after a sciatic section. These almost maximally chromatolysed cells may or may not be also vacuolated. Chromatolysis of this unilateral distribution is most evident in the last two lumbar segments. Headward of this in the L_4 segment, chromatolysis of equal severity has attacked numerous large cells scattered along the ventrolateral border of the grey matter—but on both sides of the cord. Occasional cells in a similar location in the L_5 and L_6 segments are similarly affected. Figure 9a shows one of these cells from the left side of the L_4 segment. This bilateral chromatolysis resembles in distribution that described by Cooper and Sherrington (1940) consequent on hemi- or transection of the cervical or thoracic cord,

Fig. 7–15. Untouched microphotographs of cells stained for Nissl substance. On the left, anterior horn cells; on the right, posterior horn cells. Oil immersion.

Fig. 7. Normal and vacuolated cells lying side by side in the left anterolateral group of L_7. Satellite cells have accumulated around the vacuolated nerve cell but its Nissl bodies are still clear. ×870.

Fig. 8. Large cell from the left posterolateral group of L_7, showing a minimal degree of perinuclear palor. ×870.

Fig. 9. Chromatolysis in presumed spinal border cells at L_4; a) from the left side of the completely isolated cord segment; b) from the right side eight days after a left hemisection at C_1. ×530.

Fig. 10. Cells from the right posterolateral group of L_7, showing grave chromatolysis as the result of the right sciatic section. ×530.

Fig. 11. Healthy cells in the medial-basal portion of the left posterior horn; L_7. The large cell is probably one of those composing Stilling's nucleus. ×870.

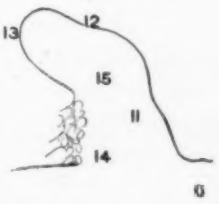


Fig. 12. Severly chromatolysed cell from the medial margin of the left posterior horn; L_7. ×870.

Fig. 13. Healthy pair of cells from the apex of the left posterior horn; L_7. ×870.

Fig. 14. Healthy cell of the type characteristic of the basal group of the posterior horn; left side; L_7. ×870.

Fig. 15. Chromatolysed cell lying just ventral to the substantia gelatinosa in the nucleus of the posterior horn of the left side. L_7. ×870.

and interpreted by them as evidence that these anterior horn cells, so-called spinal border cells, give rise to a decussating, ascending system which they believe to be Gowers' tract. Figure 9b reproduces a cell from the ventrolateral part of the anterior horn of the L_4 segment on the side opposite a hemisection at C_1 of 8 days' standing which should be one of these spinal border cells.

With few exceptions, severely chromatolysed anterior horn cells fall into one or the other of these two groupings, but lesser degrees of chromatolysis are widely distributed throughout the anterior horns. Figure 8 illustrates the most prevalent condition: a perinuclear zone of pallor without displacement of the nucleus or other disorder, and of doubtful pathological significance. This condition is distributed among the anterior horn cells quite haphazardly, selecting neither the very largest nor any particular group, and altogether about half the cells are in some degree affected. Again, both cells in this condition and those free of it may or may not be also vacuolated.

In contrast with the anterior horn cells as a group, the posterior horn cells are for the most part healthy in appearance; the small cells of the substantia gelatinosa entirely so. Of the large and medium sized cells whose long ascending axons were presumably cut by the upper transection, the majority are not visibly chromatolysed, but a not inconsiderable minority show a typical, more or less grave reaction (Fig. 12 and 15), and occasional cells appear on the verge of destruction. Reviewing the various groups of sensory cells: the pericornual cells, both marginal and apical, are less often attacked than the large cells occasionally present in the substantia gelatinosa and composing a central nucleus in the dorsal horn just below, whereas sparse but conspicuous cells of different morphology, probably to be considered Stilling's group, are almost never chromatolysed unless some of the cells so gravely affected as to be unrecognizable as to type are of this group. Generally speaking, when chromatolysis attacks individuals of these groups, it tends to be severe. In contrast is the group of cells most numerously affected. This occupies the full width of the basal part of the posterior horn, extending laterally into the reticular formation, and perhaps also anteriorly to include cells in the dorsomedial group of the anterior horn which are of similar type. About a third of these cells of variable size but characteristic morphology, presumably association cells, are chromatolysed, especially the medial lying members, but the reaction is typically moderate and unaccompanied by displacement of the nucleus. Figures 11 to 15 illustrate seemingly normal individuals from these groups and two examples of chromatolysis. Vacuolation of the flagrant type attacking the anterior horn cells, does not occur in the posterior horn, nor in the dorsomedial group of the anterior horn. Figure 12 shows the modest vacuolation occasionally present in these cells.

Summary

This brief study demonstrates the feasibility of isolating regions of mature spinal cord from all ingoing nerve impulses and utilizing them for exam-

ination of various questions in neuro-anatomy and neuro-physiology. It has given a picture of the intrinsic and motor mechanism of the spinal cord cleared of posterior root and descending fibers, showing the magnitude, variety and arrangement of the intrinsic system in the monkey. Finally, it has confirmed the conclusion reached after very much longer study of isolated segments in dogs, that the mammalian cord mechanism operates only under the stimulus of arriving nerve impulses. Deprived of such excitation the cord produces no activity which reaches effectiveness in the skeletal musculature.

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FIBRILLATION IN SKELETAL MUSCLE IN RELATION TO DENERVATION AND TO INACTIVATION WITHOUT DENERVATION*

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SKELETAL MUSCLE, when left in possession of its innervation but rendered inactive for a period of months by cutting off the innervating region of spinal cord from all ingoing nerve impulses, atrophies and becomes contractured (Tower, 1937). Examined in the intact living animal, or grossly at autopsy, there is nothing to distinguish this reaction from the reaction of muscle to denervation. Examined histologically, however, the reaction differs from denervation atrophy in the failure of the subsarcolemmal nuclei either to alter their form or to proliferate. In the final stage of fibrous metaplasia they simply disappear. The motor end-plates likewise remain virtually intact until this stage. Denervation atrophy and inactivation atrophy, or atrophy of disuse, thus appear to be related but not identical processes. In the study referred to, however, the living muscle was never exposed and directly examined for the occurrence or absence of fibrillation.

Now, with increasing appreciation of the far-reaching consequences of denervation, suggested especially by the finding that with series innervations such as those of the autonomic nervous system the effects of denervation may be evident trans-synaptically (Cannon, 1939), the possibility looms that isolation of the spinal cord may have constituted a functional denervation of the skeletal muscle such that fibrillation followed. In that case the attendant atrophy could scarcely be considered to have been the result of simple inactivation. The preparation of three adolescent monkeys with isolated lumbo-sacral spinal cords as described in the preceding communication, each with an additional right sciatic section, offered an opportunity to determine whether muscle rendered inactive but left in physical possession of its nerve supply is in fact as inactive as it appears to be; or whether, like denervated muscle, it fibrillates. The prior experience of others (Matthes and Ruch, 1931; Sherrington, 1932; Cooper and Sherrington, 1933; Fulton and McCouch, 1937) with paraplegic monkeys, which has shown the liability of this species to traumatic degeneration of the sciatic nerves, makes the monkey not the animal of choice for such a test, but as the results turned out, they are significant.

The observations for fibrillation were made terminally on two of the three monkeys in which the inactivation of the hindquarters was complete. Except for a difference in sensory level amounting to one segment, the condition of these two animals during survival was in every respect similar even

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to an ulcer developing over the right greater trochanter and not over the left. In both, the skeletal musculature below the level of lesion was atonic, completely paralysed, and uncontractured. To what extent the muscles could be considered atrophied at the time of death is uncertain for both animals had lost weight, the legs losing more in bulk than the arms. Certainly, however, both animals and both legs of each animal shared in the loss fairly equally, and the loss was not yet great.

The first indication that the condition of the two animals was not identical came to light only in the terminal examination. On the fourteenth day after the cord operation, the thirteenth after the sciatic section, muscles were exposed on the front and back of the thigh and lower leg and the interosseous muscles of the foot, and examined with a hand-lens for fibrillation. No anaesthesia was required for this procedure. In the first animal examined, the muscles of both sides were found to be fibrillating actively from thigh to foot. The sciatic nerves of the two sides were then exposed, the right in the thigh, the left near the greater trochanter. Both nerves were swollen and yellowish, and electrically inexcitable (60 cycles; 8 V). It was concluded therefore, that both nerves were degenerated, the left probably in consequence of degeneration of the isolated cord segment. This conclusion was verified at autopsy when the segment was found to be completely necrotic.

In the second monkey, similarly examined, the muscles of the right leg were similarly fibrillating from thigh to foot with the exception of those on the anterior surface of the thigh, but the muscles of the left leg were entirely at rest. The fibrillating muscles of the right side were strikingly deeper red in color than the corresponding quiet muscles on the left, and deeper red on the whole than those of either arm. The right sciatic nerve, exposed in the thigh, was swollen, yellowish and electrically inexcitable whereas the left nerve, exposed near the greater trochanter, was of normal appearance, and when stimulated (60 cycles; 1 V), caused the muscles of the calf and foot to contract quickly and strongly. It was concluded that the isolated cord segment in this animal must be in good condition, a conclusion which was again confirmed by autopsy and subsequent microscopic examination of the cord. A cross section from this isolated region of cord stained with a silver stain, may be examined in the preceding paper, together with specimens of the anterior horn cells of the two sides stained for Nissl substance.

Cutting the spinal cord off, literally, from all ingoing nerve impulses constitutes, therefore, neither a denervation of the isolated cord tissue such that this becomes spontaneously active, nor a functional, trans-synaptic denervation of the dependent and still anatomically innervated skeletal muscle such that this enters into fibrillation. The apparent quiescence in both cord and muscle seems to be real. If this be true, then the conclusion previously reached is valid—that the atrophy described under similar conditions previously is atrophy of inactivity, or so-called atrophy of disuse. By this should be understood, however, only that inactivity is the setting

for the atrophy, the mechanism of which remains at the present time still to be analysed.

The problems presented by the atrophies of skeletal muscle become more and more challenging. The apparent inactivity of denervated muscle, which had much to do with developing the general concept of atrophy of disuse, has been shown to be unreal. Denervated muscle is ceaselessly active in the minute contractions of fibers or of parts of fibers called fibrillation, and this activity continues certainly for a year (Tower, 1939a) and possibly for many years after the denervation, so long, probably, as the muscle survives as a contractile tissue. Reviewing recently (Tower, 1939b) the evidence bearing on the mechanism of denervation atrophy, weight of evidence seemed to support Langley's view, that denervation atrophy, far from being a consequence of disuse, is a consequence of exhaustion of the muscle by the continuing fibrillation. Since then, however, Solandt and Magladery (1940; in part confirmed by Ravin, 1940) have succeeded in preventing the fibrillation of denervation by the use of drugs, and have shown that the atrophy continues. Is this, then, atrophy of disuse? And have the two conditions a common basis even though atrophy of inactivity appears the simpler histologically (Tower, 1937; Tower, 1940)? Such a common basis might be sought in disturbance of an optimal tension as was long ago suggested. Or more in line with current thought, it might be sought in the cessation of neuromuscular transmission which follows equally on section and degeneration of the nerves and in circumstances of total inactivity. The neurotrophic influence thus brought to an end might conceivably be either a simple conditioning effect, or the result of substance liberated; the latter possibly, but by no means necessarily identical with the humoral transmitter, acetylcholine. Carried further for a longer survival period, though preferably not on the monkey, the experiment here described, with denervation of one extremity and inactivation of the other, may offer an opportunity to compare the morphology, physiology and chemistry of the two atrophic processes in skeletal muscle, with results of consequence for the understanding of atrophy.

Summary

To investigate the possibility that surgical isolation of the spinal cord from all ingoing nerve impulses might constitute a functional denervation of the dependent skeletal muscle such that this enters into fibrillation, the lumbo-sacral region of cord was so isolated in two monkeys and in addition, one sciatic nerve was cut in each. Two weeks thereafter leg muscles were exposed on the two sides and examined for fibrillation. Muscle still in possession of its innervation was found to be at rest, not fibrillating; but muscle denervated either by sciatic section, or, in one animal, in consequence of complete degeneration of the isolated cord segment, was found to be fibrillating and of a deeper red color than the muscle at rest. It is concluded,

therefore, that atrophy developing under conditions of inactivation without denervation may properly be considered inactivation atrophy, or atrophy of disuse.

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INITIATION OF MUSCLE IMPULSES AT NEURO-MUSCULAR JUNCTION

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A PREVIOUS PAPER (Eccles, Katz and Kuffler, 1941) has dealt with the endplate potential (henceforth called e.p.p.) set up by a nerve volley in completely curarized muscle. It has there been shown that a spike potential, due to a propagated muscle impulse, arises when the e.p.p. is caused to surpass a certain critical value, either by allowing some recovery from the curarization, or by summation of the e.p.p.'s set up by two or more nerve volleys. It is thus clear that in partly curarized muscle the e.p.p. forms an essential link between the nerve impulse and the initiation of the muscle impulse. In this paper the relationship of e.p.p. to initiation of muscle impulses will be examined for normal muscles. This investigation would be best made on an isolated single nerve-muscle fibre preparation, but, as this does not at present seem feasible, we have used the innervated strip preparation of the cat's soleus, and have also performed some parallel experiments on the frog's sartorius.

The experimental procedures have already been described (Eccles and O'Connor, 1939; Eccles, Katz and Kuffler, 1941). Special care has been taken to maintain constancy of temperature, which has often been within 0.2°C. for hours. However it will be seen that some curare experiments proved difficult in this respect, as curarization is associated with a considerable fall in the cat's metabolism, and hence with the fall of a previously stabilized temperature. The necessary detailed analysis has been obtained by photographing at high speed (up to 27.5 m. per sec.) and at high amplification, and the curves have been measured with a lens to 0.05 mm. and plotted at 20 times magnification.

A. EXPERIMENTAL RESULTS

In locating the endplate zone on an innervated muscle strip of the cat's soleus, the "recording" electrode is moved up or down the strip until the latency of the spike peak is at a minimum (usually about 1.5 msec.). Then slight sideways movements to the position for maximum spike potential ensure that the electrode is placed centrally on the endplate zone of the strip. In preparations with a sharply localized endplate focus the action potential so recorded rises steeply to a summit which passes over to a less steep decline, thus resembling in its general course the nerve spike potential. However considerable differences are observed in the initial part of these muscle potentials: in some preparations they begin abruptly with a very brief initial "foot" (Fig. 1a)—the "simple-spike" type; in others there is an initial step clearly separated by an inflexion from the steep rising phase of the spike (Fig. 1b)—the "double-step" type; finally Fig. 1c shows an intermediate

type where an initial slow rise is not clearly separated from the steep rise of the spike.

There are two probable explanations of the initial step shown in Fig. 1b and c: it may be the spike potential of a small group of muscle fibres whose endplates are nearer to the recording electrode than the main focus of endplates; alternatively the initial step may be e.p.p., which in these experiments would attain a considerable size before the spike is set up. Three tests have been employed in distinguishing between these alternative explanations, *i.e.* between e.p.p. and spike, and also in investigating the difference between the double-step and the simple-spike potentials.

(i) The effect of graded doses of curare. The spike is delayed and eventually abolished, while the e.p.p. is progressively diminished but not delayed.

(ii) The changes in the action potential produced by small shifts of the recording electrode from the endplate focus along the strip. The spike potential shows a time shift due to conduction with but little alteration in size, while the latent period of the e.p.p. is but little altered and it rapidly decrements.

(iii) Determining by subtraction (cf. Eccles and O'Connor, 1939) the potential added by a second nerve volley at various intervals after the first volley. An early second volley sets up an e.p.p. uncomplicated by muscle spike, and at longer volley intervals the spike arises from the initial e.p.p., all transitions to the normal action potential being observed.

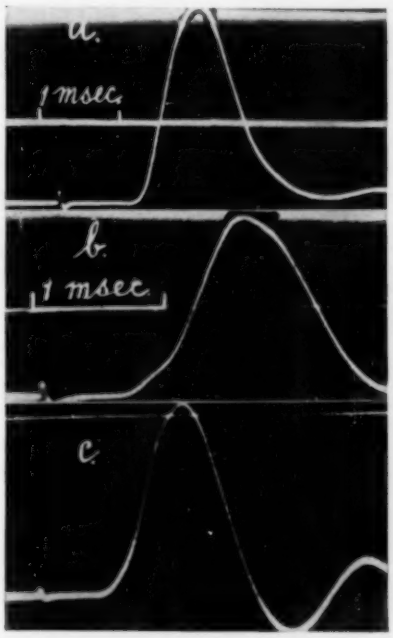

FIG. 1. Cat's soleus: action potentials which a nerve volley sets up at the endplate zone of an innervated strip in three different experiments. Time scale of c same as a.

1. *Graded curarization.* It has already been shown that in curarized muscle both the latent period and time course of the e.p.p. are not significantly altered when it is greatly depressed by further curarization (Eccles, Katz & Kuffler, 1941). This suggests that in normal muscle a similar latent period and time course would also obtain for that part (if any) of the e.p.p. which precedes the advent of the spike, and should also be observed at all intermediate stages of curarization—subparalytic, partly paralytic and paralytic.

A rough preliminary comparison may be made by superimposing on the normal action potential the e.p.p. set up in complete curarization, the respective amplifications being chosen to facilitate matching of the initial step with the e.p.p. This comparison (Fig. 2) reveals a striking similarity with

both latent period and time course. A more detailed comparison has been made by plotting on the same time-deflection co-ordinates the action potentials set up by a single nerve volley at all grades of curarization (Fig. 3). The normal action potential at 13.2 times less amplification is also plotted so as to show its initial phase of similarity with the pure e.p.p. (cf. Fig. 2b). In order to establish a relationship between the e.p.p. and the initial step, we must show that the time courses of the curves are similar, and we must also determine if there is a time shift of the curves due to any alteration which curare might produce in the latent period. This is best done as follows.

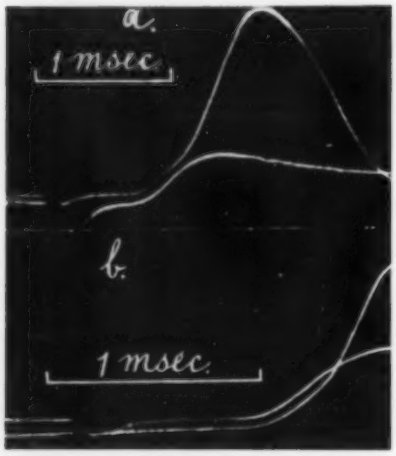

FIG. 2. Superposition of the normal action potentials of Fig. 1b and c (Fig. 1c taken with a faster time base) on the e.p.p.'s set up after complete curarization. These latter potentials are recorded at 9.3 and 13.2 times the amplification in order to give approximate matching with the initial step of the normal potentials. The stimulus artefacts are synchronized and the records are shown slightly separated to facilitate comparison.

On one curve an arbitrary point is chosen, *e.g.* B in Fig. 3, and the time BA is measured for doubling this potential, BC for halving it, etc. On each other curve points $\beta\alpha$ are chosen which by trial have the same doubling time BA (0.083 msec.). It will be seen that on the curves where this is feasible the β points lie at about the same time as B. Furthermore, if from these β points the γ points be found for the respective half potentials, these γ points again lie close to the time of C on the original curve —similarly for δ points, etc. In this way the initial parts of the lower series of curves in Fig. 3 are shown to have similar curvatures at any given time; they can be accurately matched by suitable ordinate scaling. Similarly, standardizing by the doubling time $\gamma\beta$ (0.055 msec.), the highest curve of this first series matches with those curves too high for the determination of a doubling time of 0.083 msec. In this way by using, as it were, relays in the matching, the initial parts of the whole family of curves can be shown to be accurately matchable simply by choosing suitable ordinate scaling. In Fig. 3 the extreme range of the variations in latency is only about 10 μsec. and there is no systematic change with curarization.

This matching provides strong presumptive evidence that the initial phase of all these curves is an e.p.p., which is diminished in size by curare, but not altered in latency—exactly as occurs in completely curarized muscle. The normal action potential at low amplification (curve ABC) supports these conclusions, for just beyond A it deviates sharply from the e.p.p. curve (shown by the broken line), a deviation which presumably is due to the muscle spike. Thus in this experiment it would seem that the normal action potential is initially an e.p.p., and, when this potential attains a

value about 13 per cent of the peak of the muscle action potential (henceforth called the peak-potential), the muscle spike is set up giving a suggestion of a double step. The amplification is too high to show the origin of the spike in the other records of Fig. 3.

The spike origin is well seen in Fig. 4, which gives a similar family of curves for another double-step experiment in which matching is also possible

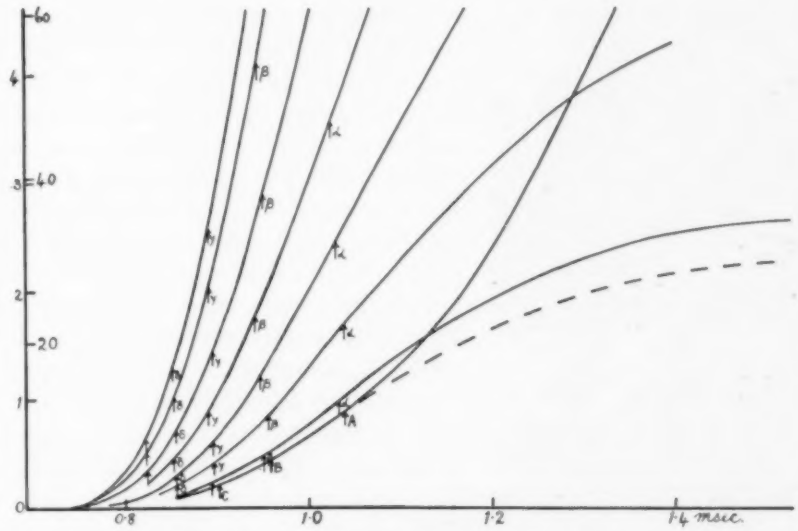

FIG. 3. Rising phases of action potentials set up at endplate zone by a nerve volley during progressive curarization, the highest curve being the normal response. All are plotted on the same time scale and the potential scale (percentages of normal peak-potential) shown to the left obtains for all except the lowest record, which is a normal action potential at 13.2 times less amplification (cf. Fig. 2b). The two next lowest curves are pure e.p.p.'s, and the broken line gives the same time course for the initial endplate potential of the normal curve, the deviation due to the spike origin being well shown. See text for the significance of the letters in relation to the detailed matching of the curves.

by choosing suitable multiplication factors. In addition to showing a constant latency of the e.p.p., Fig. 4 indicates that *pari passu* with progressive curarization there is a lengthening latent period of the spike. This lengthening is best measured by the shock-peak times. Thus in Fig. 4 the normal shock-peak time of 1.45 msec. is lengthened to 1.57 msec. with a subparalytic dose of curare, to 1.80 msec. with a considerable paralysis, and finally to 2.0 msec. with the last muscle fibre to resist paralysis. In every experiment the maximum lengthening of this shock-peak time has exceeded 0.25 msec. (cf. Fig. 5).

One other "double-step" experiment has given a family of curves similar to those of Fig. 3 and 4, and in another the test described for Fig. 3 revealed a slight temporal shift with progressive curarization—in all about 0.06 msec. Probably this shift was due to slight cooling rather than to an exceptional action of curare in delaying the time course of the e.p.p. A cooling of 0.6°C.

would account for this shift, and, as the cat's temperature fell by 1.5°C. while the box temperature did not vary more than 0.2°C., such a cooling of the muscle possibly occurred.

Thus in these four experiments there is good evidence that the e.p.p. has formed the initial step, the relatively late origin of the spike giving the main potential wave—a conclusion fully confirmed in sections 2 and 3. When the initial step of the action potential is due to an early spike response of a few muscle fibres—the alternative explanation suggested above—progressive curarization delays and eventually abolishes both the initial step and the main spike, leaving only the small e.p.p. electrotonically transmitted from the endplate zone (cf. Eccles, Katz and Kuffler, 1941). The deviation from the continuous matching series shown in Fig. 3 and 4 is so striking that such action potentials are easily distinguishable from the true double-step potentials with their initial e.p.p. step.

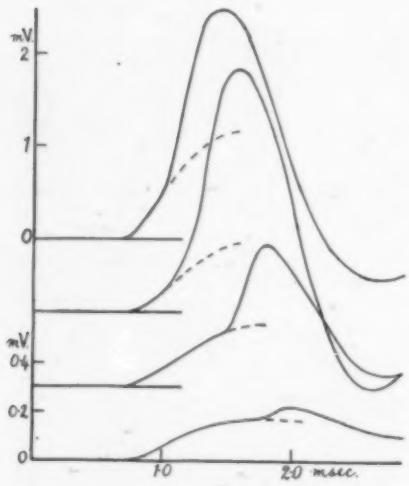

FIG. 4. Potentials set up by a nerve volley at endplate zone, plotted to show progressive delays of spikes with increasing curarization (top record normal). The potential scales for the two upper records and the two lower records are shown separately. The broken lines indicate the continuation of the initial e.p.p. step.

Figure 5 illustrates the effect of progressive curarization in a "simple-spike" experiment (observation 1). Subparalytic curarization delays the muscle spike, which now seems to take off from an initial step (observations 2 and 3). With deeper curarization (observations 4 to 9) the spike is further delayed and the initial step more obvious. Finally observations 10 and 11 show that the time course of the pure e.p.p. corresponds closely to that of the initial step, and this is seen more clearly in the corresponding plotted curves of Fig. 6. Matching tests such as those applied to Fig. 3 reveal that the initial rising phases of observations 3 to 11 (Fig. 5) match with those of observations 1 and 2, but have a latency about 0.05 msec. longer. This lengthening was due to temperature change, for it occurred while the temperature fell about 0.4°C. and the curarization was constant. Hence these matching tests indicate that the initial step in the partly curarized potentials has the same latency and time course as the e.p.p., and even the normal action potential seems to have a slight initial phase of e.p.p.

The smallness of this initial step would thus provide the only difference between this simple-spike experiment and those double-step experiments illustrated in Fig. 1, 2, 3 and 4. Whereas in Fig. 5 and 6 the spike deviation from the e.p.p. normally seems to occur when this has only attained about 1 per cent of the peak-potential, it occurred at the much higher values of 6.5,

12, 13 and 15 per cent in the 4 double-step experiments. However another experiment was transitional, for a clear double-step was observed though the spike arose when the initial e.p.p. had only attained 3 per cent of the peak-potential. Four other experiments have conformed to the type of Fig. 5 and 6, matching tests indicating a spike origin when the initial e.p.p. had attained no more than 1–2 per cent of the peak-potential.

In all these experiments the matching tests at high amplification allow us to measure the steepness of the initial phase of e.p.p. relative to its steepness in completely curarized muscle. It is then possible to calculate the maximum to which the e.p.p. would normally rise if the spike did not supervene. Table I, column 2 shows that with one exception this maximum lies between 30 and 42 per cent of the peak-potential and that there is no significant difference between experiments with large and small initial phases of e.p.p.

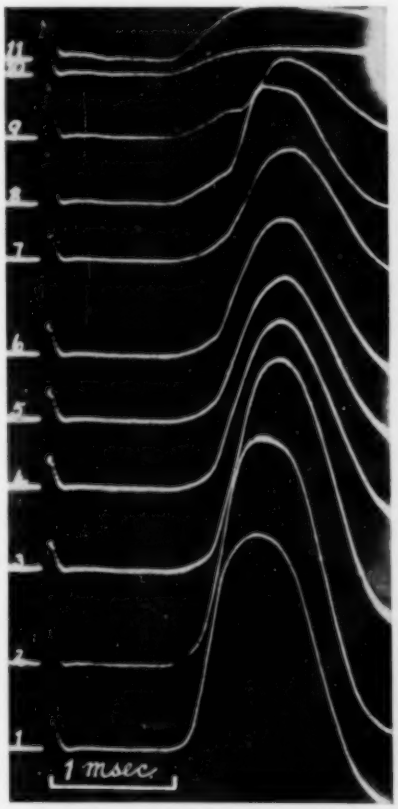

FIG. 5. Observation 1 is normal action potential at endplate zone (simple-spike type) and series shows effect of progressive curarization; 11 is the pure e.p.p. at 2.7 times amplification of observations 1 to 7, and 8, 9 and 10 are 1.7 times.

Finally there were two exceptional "simple-spike" experiments (cf. Fig. 1a) in which the matching tests failed. Thus, in Fig. 7, observations 1, 2 and 3 cannot be matched with the initial e.p.p. step which appeared in the deeper curarization of observations 4 to 6, where matching was possible as in Fig. 3. The origin of the spike is clearly seen in observation 5, and the matching test reveals its origin in observation 4. If observations 1, 2 and 3 begin with an e.p.p., then the initial dose of curare must have lengthened its latency and also altered its course, effects which curare does not have when the e.p.p. is certainly recognizable in this and other experiments. It is therefore probable that, in these first 3 observations of Fig. 7, the spike arose before the e.p.p.; a small initial e.p.p. step (about 1 per cent peak-potential) first appeared when this spike origin was delayed about 0.1 msec. by curare. With further curarization this experiment resembled that of Fig. 5. However it is not possible to exclude an exceptional action of curare in lengthening the latency of the e.p.p.

Conclusions. Detailed study of the effects of progressive curarization on the rising phase of the normal action potential provides good evidence that two distinct actions are involved.

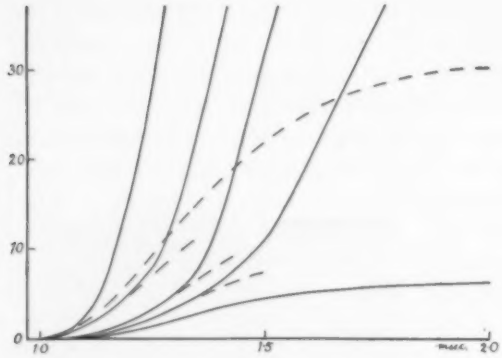

FIG. 6. Plottings of observations 1, 2, 3, 7 and 11 of Fig. 5 on the same potential-time coordinates. Ordinate scale: percentages of normal peak-potential. The continuations of the *initial* e.p.p.'s are shown by the broken lines in order to indicate spike origins.

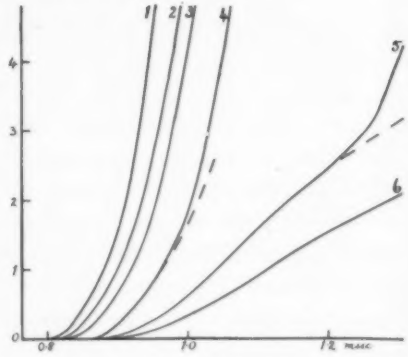

FIG. 7. Plotting as in Fig. 6 of another experiment in progressive curarization. The first three curves cannot be matched by any multiplication of the e.p.p. Temperature maintained constant throughout series: cat 39.0°C., box 38.0C°.

(a) Progressive diminution of the e.p.p. without altering its latency or time course, *i.e.* this part of the potential is matchable by suitable ordinate scaling.

(b) Progressive lengthening of the spike latency by at least 0.25 msec., and its eventual diminution and extinction.

On this basis our experiments fall into three groups. (a) In five the e.p.p. forms the initial step which attains from 3 to 15 per cent of the peak-potential before the spike supervenes. (b) Five other experiments resembled group *a* in having an initial phase of e.p.p., but the spike supervened before this had attained 2 per cent of the peak-potential, and no initial step was observed. (c) Finally in two experiments the spike appeared to arise before the e.p.p.

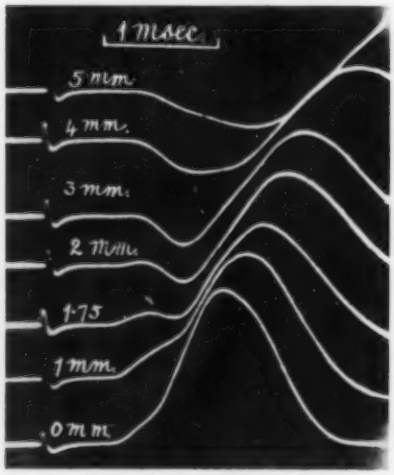

FIG. 8. Action potentials set up by single nerve volley and recorded at the endplate zone (as located by initial test), and at the indicated distances in millimetres along the strip from this zone. The shift has a differential effect on e.p.p. and spike.

With groups *a* and *b* there is no significant difference in the rate of rise of the initial e.p.p.; hence they are only arbitrarily distinguishable according as the initial e.p.p. is or is not large enough to give a step which is differentiated by an inflection from the later spike origin. Moreover a little curare converts group *b* into group *a*, and even group *c* into *b* and then into *a*, the distinctions merely arising according to the earliness of the spike origin relative to the e.p.p.

2. *Shifts of the recording electrode along the muscle strip.* In Fig. 8 the recording electrode has been moved along the innervated strip to positions successively more proximal than the endplate focus. Correspondingly the spike potential is progressively more delayed. Figure 9 shows a direct proportionality of the shift (either proximal or distal) to lengthening of the spike latency and indicates a velocity of 3.2 m. per sec. for propagation of the muscle impulses from the endplate zone in both directions. On the other hand the initial e.p.p. step shows no alteration in latency with increasing shifts, but it diminishes in size and eventually seems to disappear beyond 3 mm. from the endplate zone. This was confirmed by records at five times greater amplification. However, when the recording electrode is 1.75 mm. or more from the endplate zone, the muscle impulses arising there given an initial phase of positivity (cf. Eccles and O'Connor, 1939, p. 49). With the 1.75, 2 and 3 mm. positions this initial phase of reverse spike recording cuts into the e.p.p. giving a sharp positive dip between it and the spike. Beyond 3 mm. the e.p.p. itself shows the reverse recording already described (Eccles, Katz and Kuffler, 1941), adding its positivity to that of the spike. There is a similar gamut of changes when the recording electrode is moved from the endplate zone distally along the muscle strip. For example in Fig. 9 the arrows proximal and distal to the endplate zone mark the points beyond which the positive dip would cross the zero potential line. The action potentials of Fig. 8 are not significantly changed by altering the position or area of contact of the relatively indifferent electrode.

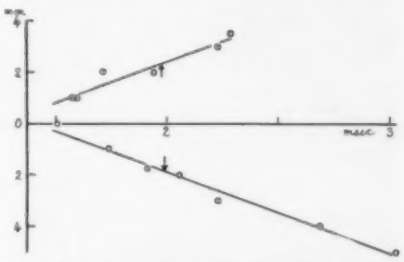

FIG. 9. Distances proximal and distal from endplate zone plotted as ordinates against latent periods of spike crests as abscissae. The oblique lines give a velocity of 3.2 metres a second, and suggest that the centre of the endplate zone is about 0.2 mm. distal to the point determined by initial test. The proximal points are plotted below the endplate line and are derived from Fig. 8.

Two other double-step experiments have given series of potentials differing from Fig. 8 only in having about half the spatial scaling. Thus in the double-step experiments the e.p.p. does not reverse sign until the recording electrode is moved from the endplate zone to about twice the distance required for reversal of spike potential. This difference appears to conflict with the conclusions that the e.p.p. is a depolarization of the muscle membrane and that it spreads electrotonically along the membrane similarly to the foot preceding the muscle spike (Eccles, Katz and Kuffler, 1941). However during the spike the great diminution in membrane impedance (Cole and Curtis, 1939) presumably would alter the circuits responsible for the reverse recording, and so may diminish the distance at which reversal of the potential is observed.

Whatever the explanation, the difference in the critical distances for reversal is useful in distinguishing between the spike and the e.p.p. Thus in

Fig. 5 graded curarization indicated that the spike supervened when the e.p.p. had attained only about 1 per cent of the peak-potential; correspondingly a shift of only 0.75 mm. from the endplate zone sufficed to give a small initial positive deflection due to inverse recording of the spike, which was so early relative to the e.p.p. that no initial phase of e.p.p. negativity could be detected. With graded curarization, however, the spike origin is delayed (cf. Fig. 5), and correspondingly 0.75 mm. away a negative e.p.p. is then seen preceding the spike, its height being about 40 per cent of that at the endplate zone.

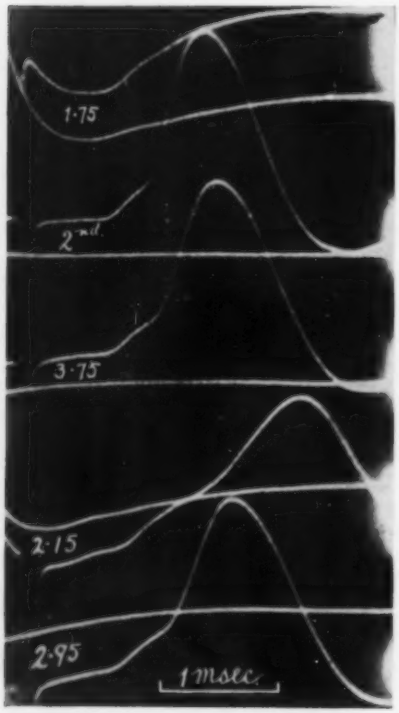

FIG. 10. Action potentials added by second nerve volley at the indicated intervals in milliseconds after the first nerve volley. The base line formed by the response to first volley alone is shown just above or below the double response. The second stimuli are fired at a constant position on the sweep. The spike origins from the initial e.p.p. steps are well shown; no spike with the 1.75 msec. interval.

Successive small shifts of the recording electrode along the innervated strip, therefore, distinguish between the e.p.p. and the spike potential and support the conclusion derived from the curarization experiments—that in some experiments a large initial e.p.p. step precedes the spike, while in others this initial potential is small or possibly absent. This method of investigation is also important in identifying the spurious initial step which is produced by the spike potential of a small focus of endplates nearer to the recording electrode than the main focus; the spurious step shows time shifts due to alterations in conduction time.

Apparent double-step potentials were frequently observed in frog's sartorius, but small shifts of the recording electrode showed invariably that both steps were due to propagating spikes arising from two discrete points on the muscle. In many other cases simple spikes were recorded at the endplate zone (see Katz and Kuffler, 1941, Fig. 1, 2, 3, 4, 8, 9, 10. Schaefer and Haass (1939) claim that double-step action potentials are always present at the endplate zone of a frog's sartorius and attribute the first step to the e.p.p. However they have not based this identification on any tests such as the three described in this paper, and, moreover, their first step is so large (up to 80 per cent of the peak-potential) that it is very unlikly to be an e.p.p preceding the spike origin. Presumably, as with the similar potentials of our frog's sartorii, their double-step potentials arise on account of two adjacent foci of endplates.

3. *Rising phase of action potential set up by an early second nerve volley.* It has already been shown (Eccles & O'Connor, 1939) that an early second nerve volley sets up a pure e.p.p., and that with longer volley intervals a spike also arises, all transitions being observed to the action potential set up by a single nerve volley. Such a series of observations form the basis of the present test for distinguishing between the rising phases of the e.p.p. and the spike in the normal action potential. The requisite degree of accuracy is obtained by employing faster recording and higher amplification as shown in Fig. 10 for a "double-step" experiment.

The potential added by the second nerve volley at each volley interval is obtained by subtraction (cf. Eccles and O'Connor, 1939, p. 65) and plotted on the same potential-time coordinates (Fig. 11), the second stimulus

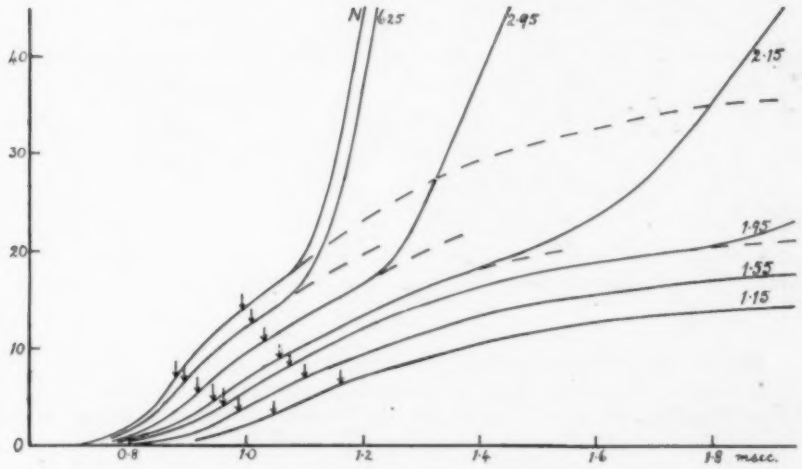

FIG. 11. Potentials (as percentages of normal peak-potential) added by second nerve volley to first response at the volley intervals indicated in milliseconds (N is normal response). With 1.15 and 1.55 msec. intervals, the added response is an e.p.p., but with longer intervals spikes are also set up later (cf. this same series in Fig. 10). The initial e.p.p. steps are continued as the broken lines. Between the arrows each curve doubles in height in 0.115 msec. (cf. matching test of Fig. 3). Other parts of the e.p.p. curves conform with this matching.

artefact giving zero time. The presence of all transitions and the general similarity of time courses strongly suggest that the e.p.p. which alone is set up at short volley intervals is also alone responsible for the initial step of the normal action potential in Fig. 10 and 11. Now the matching tests already used in Fig. 3 show that the curves of Fig. 11 can be matched provided that allowance is made for the longer latencies at the shorter volley intervals (cf. Eccles & O'Connor, 1939, Fig. 22). Thus the initial phase of all the potentials is a pure e.p.p. and the spike can be seen to arise abruptly from it, the spike latency diminishing to the normal value with lengthening of the volley interval.

The maximum height of the e.p.p. set up by an early second volley can be directly measured and expressed as a percentage of the normal peak-potential; hence the matching of the potentials makes it possible to calculate the height to which the initial e.p.p. step of the normal action potential would attain if extrapolated beyond the spike origin (cf. the upper broken line of Fig. 11). The value of 36 per cent of the peak-potential thus calculated for Fig. 11 is in satisfactory agreement with the value of 42 per cent obtained by the graded curarization test later in this experiment. Similarly, in two other double-step experiments, the responses to any second volley match closely with the initial step of the normal action potential, and are in good agreement with the effects of graded curarization.

The size of the e.p.p. may be plotted against the corresponding stimulus interval, or, more significantly, against the response interval (Fig. 12),

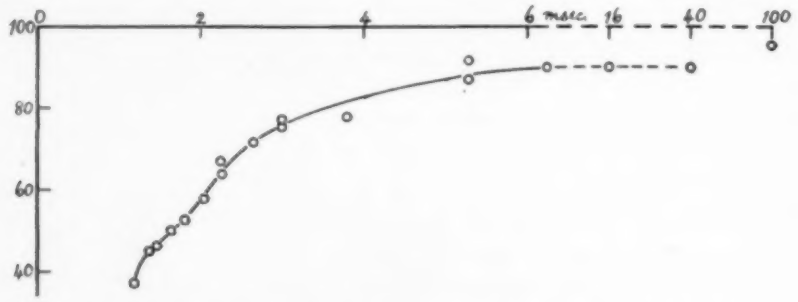

FIG. 12. Experiment partly shown in Fig. 10 and 11. Ordinates, size of e.p.p. set up by 2nd nerve volley as percentage of normal e.p.p.: abscissae, response intervals, *i.e.* intervals between corresponding points (see arrows, Fig. 11) of e.p.p.'s set up by 1st and 2nd volleys.

allowance being made for the lengthened latencies (as indicated by arrows in Fig. 11). With short volley intervals the e.p.p. is diminished to less than half its normal size and recovery is largely complete when this interval is lengthened to 5 msec.

In Fig. 13 the potentials set up by a second nerve volley in a simple-spike experiment have been plotted as in Fig. 11. Again at the shorter volley intervals there is accurate matching of the pure e.p.p.'s, but with longer intervals the early spike origin restricts the matched region to a small initial part of the curve. Thus with the normal action potential the spike arises when the e.p.p. is not more than 3 per cent of the peak-potential, and the rate of rise of this initial e.p.p. indicates that, if extrapolated, its maximum would attain 27 per cent of the peak-potential. There is satisfactory agreement with the test by graded curarization, which indicated that there was an initial e.p.p. phase of about 2 per cent and an "extrapolated maximum" of 35 per cent.

Similarly in three other simple-spike experiments matching of the added e.p.p.'s showed that the spike was set up after an initial e.p.p. phase of

about 2 per cent and there was an "extrapolated maximum" of 26 to 30 per cent of the peak-potential.

The matching of the curves of Fig. 13 allows the size of the e.p.p. to be plotted against the response interval just as in Fig. 12. The curve so obtained (Fig. 14) closely resembles that of Fig. 12, and other experiments of this type have also given similar curves. This similarity confirms the general conclusion derived from the graded curare tests—that the experiments giving normally action potentials with the simple-spike and double-step type are only arbitrarily distinguishable according as the initial e.p.p. is or is not large enough to form a discrete step.

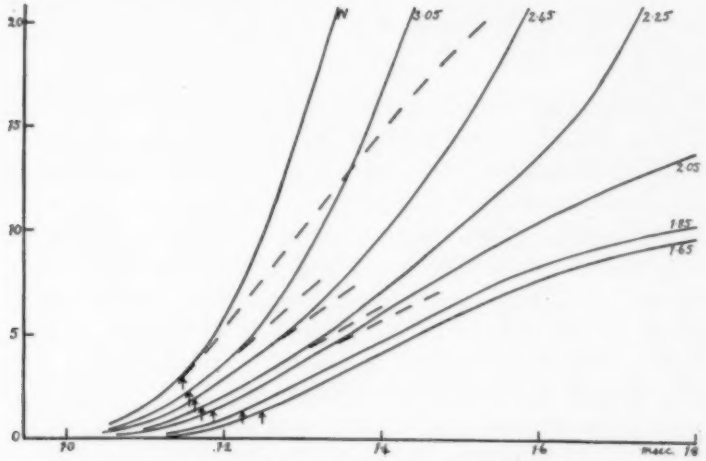

FIG. 13. As in Fig. 11, but in a simple-spike experiment. The arrows mark corresponding points on the e.p.p. curves as determined by matching. The broken lines show the continuation of the initial e.p.p. phases along courses matching the pure e.p.p.'s at the two shortest volley intervals.

The curves of Fig. 12 and 14 have some theoretical significance. In another paper (Eccles and Kuffler, 1941) it will be shown that an early second nerve volley sets up a diminished e.p.p. on account of the refractoriness of the muscle—an effect similar to that observed for the e.p.p. in completely curarized muscle (Eccles, Katz and Kuffler, 1941). The responses of completely curarized muscle show that, in the absence of muscle refractoriness, a second nerve volley more than 1.5 msec. after the first has a practically constant excitatory action in setting up an e.p.p. (Eccles, Katz and Kuffler, 1941, Fig. 17a). Hence the curves of Fig. 12 and 14 express recovery from the muscle's refractoriness, as measured by its ability to respond by an e.p.p. to the approximately constant excitatory action of the second nerve volley.

Figures 12 and 14 show that, 1.5 msec. after the response to the previous volley, a second nerve volley sets up an e.p.p. only 30–45 per cent of the normal value. There is a suggestion of an initial phase of slower recovery up to about 1.8 msec., and then recovery of the muscle is practically linear

to 3 msec., where the response is 65–75 per cent normal. At 6 msec. recovery may be almost complete (Fig. 14), or a small deficiency may remain for some time—up to 100 msec. in Fig. 12. This prolonged deficiency recalls the diminished e.p.p. set up by a second volley in curarized cat's muscle (Eccles, Katz and Kuffler, 1941), but the recovery time is there very much longer—3 to 10 sec.

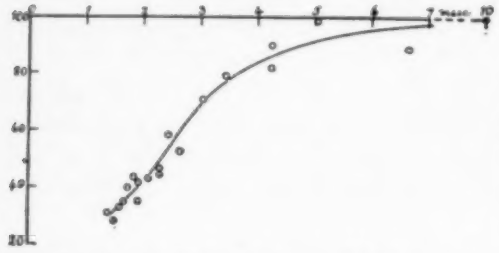

FIG. 14. As in Fig. 12, but for the experiment partly shown in Fig. 13.

B. DISCUSSION

From the three tests described above it is clear that all transitions exist between experiments in which the normal action potential shows a large initial e.p.p. step (12 to 18 per cent of the peak-potential) and those where the spike is initiated so soon that the e.p.p. has risen to not more than 1–2 per cent of the peak-potential—or even possibly has not risen at all. Further, these latter simple-spike types are converted into the double-step type either with subparalytic curarization (Figs. 5, 6 and 7) or with the action potentials set up by the earliest second nerve volleys to initiate spikes (Fig. 13).

As shown in columns 2, 3 and 4 of Table 1, the extrapolated heights of the normal e.p.p. and the fractions to which it is diminished with just subparalytic ($\frac{2}{3}$ to $\frac{2}{5}$) and half-paralytic curarization ($\frac{2}{5}$ to $\frac{1}{5}$) are consistent and show no significant relationship to the size of the initial phase of e.p.p. (column 5), *i.e.* to the simple-spike or double-step type of action potential.

When the initial e.p.p. is large (12–15 per cent of the peak-potential—early in experiment 18/4/40 it was 18 per cent), progressive curarization is associated with a spike origin at lower e.p.p.'s (columns 6 and 7), and complete paralysis (column 8) is not produced until the summit of the e.p.p. is less than 40 per cent of the initial e.p.p. step (cf. Fig. 3 and 4). It is probable that the actual threshold potential remains approximately constant at any one endplate, because curare has little or no effect either on the electrical excitability of muscle or on the propagation of muscle impulses. However, with progressive curarization, the following factor would cause the spike to be initiated at a lower observed value for the e.p.p.

A given dose of curare will diminish to varying degrees the e.p.p.'s at different endplates. This would in part arise on account of varying concentrations of curare, as determined for example by the relation of the endplates to the blood vessels. Also in some experiments pressure of the recording electrode might seriously have interfered with the circulation to part of the endplate zone. Presumably muscle spikes would be set up first at those endplates least affected by curare, and so would arise when the aggregate e.p.p. is less than the threshold potential obtaining normally in the absence of this unequal depression by curare.

This factor is probably adequate to account for the spike origin at dimin-

Table 1. Columns 2, 5, 6, 7 and 8 are expressed as percentages of peak-potential

Experiment	"Extrapolated maximum" for normal e.p.p.	Rate of rise of e.p.p. (as fraction of normal rate)		Height of e.p.p. at spike origin			Maximun height of e.p.p.—just complete curarization
		Just subparalytic curarization	Half paralytic curarization	Normally	Just subparalytic curarization	Half paralytic curarization	
1	2	3	4	5	6	7	8
9/2/40	30			3			4.5
13/2/40	37	$\frac{1}{2}$	$\frac{2}{3}$	13	6	5	5
16/2/40	?			?0	2.5	2.5	3
23/2/40	41	$\frac{1}{2}$	$\frac{1}{4}$	6.5	4.5	2.5	3.5
27/2/40	35			1			3
7/3/40	?			?0		1.5	6
13/3/40	35	$\frac{2}{3}$	$\frac{1}{4}$ to $\frac{1}{3}$	2	1.5	2.5	2.5
15/3/40	30	$\frac{1}{2}$	$\frac{1}{3}$ to $\frac{1}{4}$	12	7	3	2.6
18/3/40	30	$\frac{2}{3}$	—	1 to 2	4		5.5
18/4/40	42	$\frac{2}{3}$	—	15	9		5.5
13/5/40	50		$\frac{1}{3}$	1.5		2.5	

ishing e.p.p.'s in columns 5, 6 and 7 of Table 1. Thus in these three experiments the e.p.p. is probably the sole mechanism initiating the muscle impulses both with normal and with subparalytically curarized endplates. On that basis the safety factor obtaining for normal neuro-muscular transmission would be approximately given by the ratio of the values of column 2 to those of column 5. Thus the safety factor for each of these three experiments is about 3. Alternatively the safety factor for the average neuromuscular junction may be derived from the diminution in e.p.p. necessary for half paralysis. Under such conditions there would be an approximately symmetrical distribution of the endplates with respect to the just paralytic intensity of curarization, and the aggregate e.p.p. would be diminished to about the degree which is critical for interrupting neuro-muscular transmission at the average neuro-muscular junction; hence the reciprocal of the diminution shown in column 4 would give the average safety margin. The values of 2.5 and 3.5 so derived for the safety margin in experiments 13/2/40 and 15/3/40 are in good agreement with value of about 3 calculated by the previous method.

On the other hand in Table 1, columns 5, 6 and 7 show that, in experiments with normally a very small initial phase of e.p.p., progressive curariza-

tion is associated, on the whole, with a spike origin at an increasing e.p.p. (cf. Fig. 5 and 6). Finally column 8 shows that with a just paralytic degree of curarization the e.p.p. is practically as large as with the double-step experiments. This progressive increase would be accentuated by the above factor. Thus these observations suggest that, at any one endplate, the e.p.p. is normally very small when the muscle impulse is initiated, and this critical size becomes increasingly larger as the curarization deepens—a condition which is improbable if the e.p.p. is the sole factor initiating the muscle impulse. Again with these experiments the above calculations of the safety factor show a wide discrepancy—a value of 15 or more being given by the first method above, and 3 to 5 by the second. Finally we have one and possibly two experiments (16/2/40 and 7/3/40) in which the muscle impulse may have been initiated even before the e.p.p.

Thus, with this group of experiments, where the initial phase of e.p.p. is normally small or even absent, there is probably some additional agent initiating muscle impulses. In other respects we have seen that these experiments appear to be similar to the above three experiments (experiment 23/2/40 is probably intermediate), *e.g.*, there is a similar extrapolated maximum for the normal e.p.p. (column 2), and a similar height for the e.p.p. at just complete paralysis (column 8); thus they differ only in that they appear to have an additional exciting agent superimposed on the e.p.p. mechanism. The increase shown in columns 5 to 6 to 7 to 8 for this group indicates that with progressive curarization the e.p.p. takes an increasing share in the initiation of the muscle impulses; hence the additional exciting agent is probably depressed *pari passu* with the e.p.p. The simplest suggestion is that the action currents of the motor nerve impulses exert this additional exciting effect, and the anatomical relationship of the nerve fibre to the motor endplate and the adjacent muscle fibre might account for the great variability from one experiment to another. Against such a suggestion is the depressant action of curare, which is not observed with direct electrical excitation of muscle fibres, though it must be remembered that the endplate zone is a specialized region of the muscle.

Thus it may be concluded that in a few experiments a nerve impulse normally initiates the muscle impulse by setting up the intermediary e.p.p., and there is no evidence for any other excitatory action on the muscle fibre. More often there appears to be an excitatory action additional to the e.p.p., and in a few experiments this additional mechanism may even be solely responsible for normal neuro-muscular transmission, the e.p.p. mechanism being revealed only when the additional mechanism is depressed by curare or by the refractory period following a previous muscle response. We have no evidence relating to the nature of such an additional mechanism, and no sign of its subliminal action can be seen. Possibly this exciting mechanism is analogous to the detonator action described for synaptic transmission (Lorente de Nó, 1935, 1939; Eccles, 1937, 1939), and it may be caused by the direct excitatory action of the nerve action currents on the muscle fibre at the region of the neuro-muscular junction.

Summary

A detailed study has been made of the rising phase of the muscle action potential which a nerve volley sets up at a focus of endplates. In some preparations these potentials are of the "simple-spike" type, in others an initial step precedes the spike (the "double-step" type).

Three procedures have been used in identifying that component of the rising phase due to "endplate potential" (e.p.p.): progressive curarization; small shifts of recording leads away from the focus; responses added by a second volley at various intervals.

It is thus shown that the initial step of the double-step potentials is due to e.p.p., the spike only arising when this has attained a considerable size—up to 16 per cent of the peak-potential. With the simple-spike potentials there is usually also an initial e.p.p. phase, but it is small—usually 1–2 per cent of the peak-potential. All transitions are observed.

There is no significant difference between the e.p.p.'s of the two types: in both the initial steepness of e.p.p. rise indicates an extrapolated maximum of about 30–40 per cent peak potential; in both curare and the muscle's refractory period depress the e.p.p. similarly. The difference solely concerns the earliness of the spike origin relative to the e.p.p.

Large initial e.p.p.'s (12–18 per cent peak-potential) are probably the sole mechanism initiating muscle impulses both normally and during subparalytic curarization or refractoriness of the muscle. The normal safety margin is about 3.

In the other experiments the excitatory action of the e.p.p. seems normally to be forestalled by an additional excitatory mechanism, which is depressed by curare or refractoriness, the e.p.p. then taking an increasing share in excitation. The nerve action currents may be directly responsible for this additional excitatory effect.

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THE HUMAN ELECTRO-CORTICOGRAM

A REPORT OF SPONTANEOUS ELECTRICAL POTENTIALS OBTAINED FROM THE EXPOSED HUMAN BRAIN*†‡

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I. INTRODUCTION

ELECTRO-ENCEPHALOGRAPHY, or the recording through the intact skull of spontaneous electrical potentials generated by the brain, has assumed during the past two or three years increasing importance as a clinical aid in the discovery and localization of intracranial disease (1, 2, 3, 4).

It seems self-evident, however, that the ultimate value of the clinical encephalogram must be based upon a secure knowledge of the electro-physiology of the underlying brain, which can only come from precise observations taken directly from the exposed brain. An appreciable amount of work has been done upon the electrical activity of the exposed cortex of animals; and these accumulated observations are quite valuable for comparative study (5, 6). However, even for the purpose of studying normal human physiology, observations made from the brains of experimental animals are no substitute for observations made directly from the human brain. Moreover, the difficulty of reproducing in laboratory animals pathological processes comparable to those found in man practically precludes animals as a source of knowledge about the electrical activity of the brain in the presence of disease,—especially the neoplastic, degenerative and convulsive diseases. For such information one must study directly the exposed brains of sick patients.

Electrical potentials recorded directly from the human brain have been reported in only a few instances (7, 3, 8). This paper describes electrical activity obtained by us directly from human brains exposed at the operative table, in patients suffering from a variety of intracranial diseases.

II. METHOD OF STUDY

Electro-corticograms were taken during eighteen operations performed upon seventeen patients suffering with a variety of organic intracranial lesions. The cortex in the cases

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‡ Case reports and figures are given in full in the reprints which may be obtained from the authors.

studied was exposed by the usual operative technique for craniotomy. Wherever possible, local anesthesia alone was used although it was necessary to do a certain number of the cases under avertin anesthesia. In a few cases it was necessary to supplement the avertin with very mild temporary inhalation anesthesia of ether. In one patient, a small child, primary ether anesthesia was used throughout. Details regarding anesthesia have been included in the individual case reports. The exposed cortex was first stimulated electrically, principal motor points marked by tags placed directly on the brain, and operative field photographed. Electro-cortical potentials of the exposed brain were then studied. In the first three cases, unipolar recording was employed; but in all of the subsequent cases, recording was made with bipolar electrodes (Fig. 1). These bipolar electrodes were made of lightweight silver wire mounted on a "mechanical stage." Fine, accurate movements in all directions could be easily and quickly made with screw adjustments; and yet at the same time complete immobility of the electrode at the time of recording was obtained. The electrode assembly was sterilized in the autoclave.

The electrodes were never allowed to pierce the pia or the cortex because the injury potentials so evoked unnecessarily would complicate the observations (10). Initial records

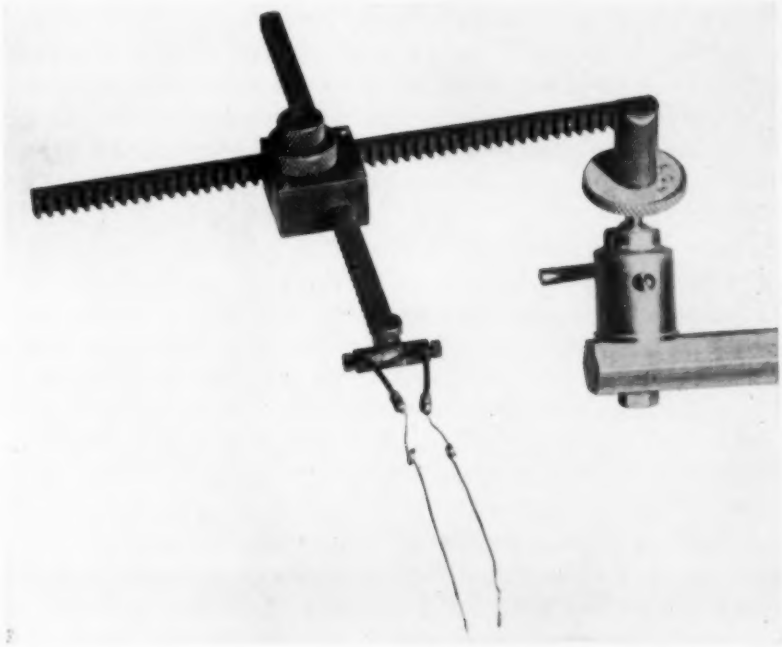

FIG. 1. The bipolar electrodes here shown were developed early in the study, and were used for recording on all except the very earliest of the cases. These electrodes were made of light, coiled, silver wire mounted on a form of mechanical stage which permitted fast, accurate adjustments in all directions.

Great care was always taken to prevent the tips of the electrodes from piercing the pia mater, since it has been shown that this is a frequent source of false potentials.

were usually taken prior to any surgical manipulation of the cortex. Subsequent records from cut surfaces of brain tissue were also made in cases which afforded this opportunity.

Shielding in the form of a single sheet of copper screen was placed under the patient during the preparations for the operation. The copper sheet and the amplifying system were both grounded. It was necessary to disconnect the patient from the electro-cautery machine prior to recording and also curtail the use of extension cords and other electrical appliances in the operating room. Records of the cortical potentials were made by means of suitable amplifiers and dynamic ink-writing recorders, mounted in a small and mobile

cabinet which was easily handled in the operating room. This equipment constructed by one of us (W.E.R., Jr.) has already been technically described (9). An accurate record was always kept of the pulse and respiratory rates during the recording and all records subsequently eliminated where synchrony existed between apparent rhythmic electro-cortical activity and these rates.

The only serious artefacts which arose were caused by gross movement of the electrodes incident to pulsations of the brain. These artefacts could in most instances be eliminated by applying the electrodes so that the points move perpendicularly with the pulsations rather than making a sliding movement over the cortical surface. The introduction of coils in the electrode arms was found to increase their flexibility and greatly reduce these artefacts. (Fig 1).

III. Observations

(A) *Classification and regional distribution of spontaneous rhythmical potentials.* In the categorical consideration of spontaneous brain potentials we have used the following classifications. Potentials having a frequency less than eight cycles per second are called "slow waves," and on the basis of empirical findings are considered to be abnormal. Those potentials ranging from eight to twelve cycles per second are considered to be alpha waves. The average frequency of the alpha rhythm is ten cycles per second. Rhythmic variation higher in frequency than twelve cycles per second are classed as beta potentials. Both alpha and beta potentials are considered to constitute normal rhythms.

In the cases we have studied, no absolutely constant localization of spontaneous rhythms could be demonstrated. It would appear, however, that the *alpha* rhythm is confined chiefly to the parietal, temporal, and occipital lobes. In only one case have we observed potentials of alpha frequency in the frontal lobes. The central sulcus appears to be the anterior limit of the alpha rhythm. Our finding of the alpha rhythm in the temporal and parietal lobes confirms the work of Berger (12), and Jasper and Andrews (13), and is at variance with the findings that the alpha rhythm is exclusively occipital in origin. The alpha rhythms ranged in frequency from eight to twelve cycles per second with an average frequency of ten cycles per second.

Beta activity is the dominant rhythm in the frontal lobes and though this rhythm may appear in the temporal or parietal lobes, when this occurs the amplitude is usually greatly diminished as compared to that in the frontal region. So that while there is a wide individual variation in the amplitude of beta potentials, this relative difference in amplitude between the frontal lobes and other portions of the brain is usually maintained. This rhythm is also more pronounced in the anterior than in the posterior portions of the temporal and parietal lobes. The observed beta rhythms showed a range of from thirteen to twenty-two cycles per second with an average frequency of eighteen cycles per second.

Illustrative examples of the *alpha* rhythm are to be found in Case I,‡ Fig. 3, graph 2; Case VI, Fig. 9D; Case VII, Fig. 10E; and Case XV Fig. 21, graph 16. Examples of the *beta* rhythm are given in Case I, Fig. 3, graph 1; Case IV, Fig. 7A; Case V, Fig. 8B and C; Case VII, Fig. 10E; Case VIII, Fig. 11A; Case IX, Fig. 12B; Case XI, Fig. 14A and B; Case XII, Fig. 15A;

Case XIII, Fig. 17B; and Case XIV, Fig. 19, graph 9. Examples of *beta* activity arising posterior to the central sulcus are found in Case VIII, Fig. 11A; and Case XII, Fig. 15A. The single instance in which *alpha* activity was obtained anterior to the central sulcus is not among the illustrative cases used in this paper.

The optic nerves anterior to the chiasm yielded rhythmic potentials of alpha frequency in one case of our series in which it was possible to place a recording electrode directly upon one of the optic nerves (Case II, Fig. 4B). Electric potentials recorded from the cerebellum, in one case (Case III, Fig. 5) were difficult to interpret, and do not permit any generalizations regarding them to be made.

Slow waves were obtained from damaged areas of the cortex regardless of the anatomical location. They appear to be merely an expression of physiological impairment, and depend for their production solely upon injury to nerve tissue. They are affected more by the degree of injury than by its nature or by the cytoarchitecture wherein the injury occurs.

(B) *Tumor tissue is electrically inactive regardless of type of tumor.* The electrical activity of tumor tissue was investigated directly at the operating table in eight patients. The tumors studied included three glioblastomas, one astrocytoma, one meningioma, one craniopharyngioma, and two metastatic carcinomas.

In not a single instance was spontaneous electrical activity obtained directly from neoplastic tissue.

Illustrative examples of these recordings are to be found in Case IV (glioblastoma) Fig. 6 and 7B; Case V (astrocytoma) Fig. 8A; Case VI (meningioma) Fig. 9B; Case VII (metastatic carcinoma) Fig. 10D.

(C) *Abnormal electrical potentials, usually associated with brain tumors, arise not from tumor itself, but from damaged brain tissue immediately adjacent.* This is clearly demonstrated in Case IV, Fig. 6 and Fig. 7C; Case V, Fig. 8B and C; Case VI, Fig. 9D; Case VII, Fig. 10E. The infiltrating tumors (gliomas) characteristically produce severe and widespread changes, both anatomic and physiologic, in the surrounding brain, thus giving rise to electro-cortical dysfunction which is correspondingly great (Case IV, Fig. 7C; Case V, Fig. 8B and C). The maximum disturbances are observed in those regions of the cortex which are being most actively invaded by the tumor (Case V, Fig. 8B). At greater distances from the tumor the abnormal potentials are gradually replaced by the normal rhythmic activity of that part of the brain (Case V, Fig. 8C).

The encapsulated tumors (meningiomas), on the other hand, usually produce very little physiologic disturbance in the adjacent brain, with the result that these tissues usually reveal amazingly little electro-cortical dysfunction, even though the tumor itself may be quite large (Case VI, Fig. 9D).

The frequencies of the spontaneous rhythms arising from tissue in the vicinity of the diffuse infiltrating tumors are shifted downwards as compared with the frequency of those rhythms characteristic of normal tissue in the

same brain. On the other hand, the encapsulated benign type of neoplasm, by reason of the limited abnormal potential changes induced, shows no lowering in the frequency of the brain rhythms arising in the tissue adjacent to the tumor. These frequency shifts are easily seen if the alpha frequency is measured carefully in tissue near the tumor and at a distance therefrom.

It is often possible, therefore, to distinguish between the infiltrating and the encapsulated tumors not only by the magnitude of the electro-cortical dysfunction and its topographical extent, but also by the degree to which the frequency of spontaneous cortical rhythms have been slowed down.

As cortical tissue becomes increasingly damaged, however, a point is finally reached, as might be expected, where all electrical activity ceases. This is well shown in Case VII, Fig. 10B.

(D) *Changes in electric potentials in association with internal hydrocephalus.* In two patients with increased intracranial pressure secondary to craniopharyngiomas, large, slow waves were recorded from the surface of the distended dura overlying the middle frontal convolution. Following a ventricular tap by means of a brain cannula, the slow waves disappeared immediately and were supplanted by beta activity (Case IX, Fig. 12). The rapidity with which this change took place was startling.

There seemed, however, to be some variability in regard to the presence of the slow waves with the increased intracranial pressure. One of our cases was re-operated upon ten days later with a recurrence of the hydrocephalus and intracranial pressure. This time, despite a similar degree of dural distension, slow waves were only occasionally present. This finding appears to corroborate the findings of Williams (14), showing that there was no direct, or at least constant, relation between the development of increased intracranial pressure as such and appearance of slow potentials.

(E) *Electric potentials associated with focal trauma to the brain* were studied in one patient (Case X), who had a cortical scar resulting from an old depressed fracture of the skull of five years' standing. In this instance large, slow waves were obtained from the immediate vicinity of the scar tissue (Fig. 13).

(F) *Diffuse pathological diseases of the brain*, as might be expected, showed normal brain rhythms interspersed with slow waves over the entire area exposed. The differences between the electro-cortical dysfunction produced by the atrophy of arteriosclerosis (Case XI, Fig. 14A and B) and diffuse encephalopathy (Case XII, Fig. 15B) seems to be one of degree rather than of quality. There appears no characteristic change which would enable one to differentiate further in this group. The degree of electro-cortical dysfunction is roughly proportional to the extent of the pathological process.

(G) *Electro-cortical activity in association with focal clinical convulsions* was studied in four cases. All of these patients manifested sharply focal motor seizures affecting primarily a single extremity, and being confined to one side of the body. In all cases tumor of the brain and other gross cortical lesions had been first excluded by means of pneumo-encephalograms.

Widespread and diffuse electro-cortical dysfunction was revealed in the electro-corticograms taken from the exposed brains of each of these patients, in spite of the fact that the clinical manifestations in each instance were exquisitely focal (Case XIII, Fig. 16 and 17; Case XIV, Fig. 18 and 19; and Case XV, Fig. 20 and 21).

Maximum abnormality, on the other hand, did appear in each case to come from a region corresponding generally to the site of the "epileptic focus," as pre-determined upon clinical and anatomical grounds. This is well shown, for instance, in Case XIV, Fig. 18 and 19, where it is evident that the abnormal activity coming from point No. 10 is greater than that coming from other parts of the cortex. It is also clearly demonstrated in Case XV, Fig. 20 and 21. Here it is evident that the most marked electro-cortical dysfunction arises from the region of markers 8 and 9, which correspond closely to the presumed epileptogenic focus, although it is manifest that the electro-cortical activity of the entire hemisphere is abnormal.

The type of abnormal electrical activity found in these convulsive disorders consisted entirely of large, slow potentials of 3–5 cps., generally indistinguishable from the abnormal potentials associated with tumors. No "spike" formations were seen (Figs. 17–19 and 21).

The evidence contained in these four cases of focal convulsions suggests that the focal phenomena in such cases may be merely an expression of *maximum* abnormality coming from a cerebral hemisphere, which, as a whole, is functioning in an abnormal manner. An epileptogenic "focus" might be regarded, therefore, merely as an area of maximum abnormality in a brain affected by a diffuse electro-cortical dysfunction.

IV. Discussion

The central sulcus appears to be the most significant anatomical boundary for spontaneous rhythmic activity. The alpha rhythm or rhythms arise almost solely in the parietal, temporal and occipital lobes. Likewise, the beta rhythm or rhythms occur most prominently in the frontal lobes, though they may appear with greatly diminished amplitude elsewhere in the cortex. The central sulcus limits the alpha rhythm more definitely than it does the beta activity.

It might be said that the alpha rhythm seems to be allied in a very general way with sensory function. This concept is supported by the fact that all forms of sensory stimulation are capable of abolishing the alpha rhythm, a finding which would be difficult to explain if the alpha activity arose only in the optic cortex, as some authors have suggested.

Beta activity seems in the same general way to be associated with motor function. In one case of focal convulsive disorder, the beta potentials in the portion of the motor gyrus which represented, upon stimulation, the anatomical focus of this disorder, showed spasmodic prolonged outbursts of very high amplitude.

In one case it was possible to put electrodes directly on the optic nerve.

Rhythmic potentials of alpha frequency were found to be present. Gerard (15) has reported that in the cat the spontaneous rhythm found in the optic cortex is present throughout the entire optic tract. It is both interesting and significant that these rhythmic potentials should be transmitted over fiber tracts. It is possible that this type of fiber tract transmission is responsible for the foci of abnormal activity which commonly appear over the frontal lobes with cerebellar tumors (16).

The abnormal potentials which are present in the tissue surrounding a benign encapsulated tumor are probably caused by mechanical deformation of the cortex. This is shown by the fact that abnormal potentials are found only in the immediate vicinity of the tumor. Likewise, the relative paucity of abnormal potentials associated with this type of tumor suggests that although it may have greatly deformed the cortex, the change has been slow enough to allow the surrounding neural tissue to make adequate physiological adjustment.

With the infiltrating type of tumor the area of damage is large and the physiological impairment ranges from a lowering of the frequency of the normal rhythms, to complete absence of rhythmic activity. These findings seem to fit a pathological picture varying from mild cellular edema to completely degenerated tissue.

The findings in hydrocephalus are variable and difficult to understand. Apparently under certain conditions it is possible to produce generalized abnormal potentials which are rapidly reversible with the release of the increased pressure. This finding has also been demonstrated by Williams (14). The only fact that appears clearly is that the production of abnormal potentials is not related to the manometric pressure alone.

In the four patients studied, who, clinically, had sharply focalized convulsions, it was found that abnormal electrical activity was found to be widespread throughout the hemisphere. On the other hand, the region from which the most abnormal electro-cortical activity arose did correspond roughly to the site of the so-called epileptogenic focus, as determined upon clinical and anatomical grounds, and by electrical stimulation.

These few observations, therefore, suggest that a so-called epileptogenic focus may represent merely the point of maximum abnormality in a brain affected with diffuse, widespread electro-cortical dysfunction. Such a concept, of course, if sustained might lead us to revise our present thoughts relative to the surgical attack upon epilepsy. However, this subject requires much more data and study before the truth can be known.

In several cases it was necessary to deepen the narcosis by means of inhalation ether anesthesia. It was observed that the ether shortly abolished the normal spontaneous rhythms and produced large slow waves in the order of one cycle per second or less. It is quite necessary to take into consideration, therefore, both the type of anesthetic used, as well as the depth of anesthesia if the spontaneous cortical activity is to be evaluated. Our own ob-

servations did not justify us in making at this time any generalizations on this subject.

V. Summary

A compact, portable apparatus has been described and a simple technique has been outlined which permit reliable records of electrical potentials to be taken directly from the exposed brain at operation.

The regional distribution of normal spontaneous cortical potentials has been outlined. Alpha activity was obtained directly from the exposed optic nerve.

Tumors were demonstrated to be electrically inactive. This was found to be true for meningiomas, gliomas of various types, craniopharyngiomas, and metastatic tumors.

The slow potentials commonly associated with tumors were found to arise from the damaged tissue adjacent to them, rather than from the tumors themselves. The area in which these abnormal potentials occur is small with the benign encapsulated tumors and much greater with the infiltrating type.

Changes in electrical potentials in association with hydrocephalus, cortical cicatrices, cerebral atrophy secondary to arteriosclerosis, and diffuse encephalopathy have been reported.

Abnormally large, slow potentials were obtained from widely distributed points of the cortex in patients who, clinically, exhibited sharply focal convulsions. This dysfunction appeared greatest at points which corresponded roughly with an epileptogenic focus, as determined on anatomical and clinical grounds. The electro-cortical dysfunction seemed definitely greater in the pre-central areas of the brain than it did in the post-central regions. "Spike" formations were not observed.

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